VOL. VII, SEC. B, NO. 3 JUNE, 1912

THE PHILIPPINE JOURNAL OF SCIENCE

SECTION B

THE PHILIPPINE JOURNAL OF TROPICAL MEDICINE

RICHARD P. STRONG, PH. B., M. D.
EDITOR

MANILA
BUREAU OF PRINTING

PUBLICATIONS FOR SALE BY THE BUREAU OF SCIENCE, MANILA, PHILIPPINE ISLANDS

REPORT OF THE INTERNATIONAL PLAGUE CONFERENCE.

Held at Mukden, April, 1911, under the auspices of the Chinese Government.

Edited by ERICH MARTINI, G. F. PETRIE, ARTHUR STANLEY, AND RICHARD P. STRONG.

483 pages, 18 plates (2 colored, 4 half-tones, 12 charts and maps).

Order No. 416. Cloth, $3.50; paper, $2.50 United States currency, postpaid.

The proceedings of this International Conference and information gained therefrom, together with the results of certain bacteriological investigations, constitute the present report.

Nothing hitherto has been published which gives such a complete and comprehensive account of the entire subject of pneumonic plague.

Delegates from America (United States of), Austria-Hungary, France, Germany, Great Britain, Italy, Japan, Mexico, the Netherlands, Russia, and China attended the Conference.

The Bureau of Science of the Government of the Philippine Islands has been appointed sole agent for the distribution of the printed proceedings of the International Plague Conference.

THE SUGAR INDUSTRY IN THE ISLAND OF NEGROS.

By HERBERT S. WALKER.

145 pages, 10 plates, 1 map.

Order No. 412. Paper, $1.25 United States currency, postpaid.

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Tables of soil analyses, both chemical and physical; analyses of the cane, juice and bagasse; estimates based on actual information as to the costs of production and of cultivation; and estimates of the cost and location of possible central factories. The island is considered by sugar-producing districts; the area of cultivation and the production per hectare are given, and the possibility for future expansion discussed.

The plates illustrate various phases of sugar industry from the cultivation of the field to the transportation of sugar in native sailboats.

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By CHARLES S. BANKS.

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B. THE PHILIPPINE JOURNAL OF TROPICAL MEDICINE

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STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

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STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

I. INTRODUCTION. THE EXPEDITION TO MANCHURIA AND THE CONDITIONS UNDER WHICH THE WORK WAS PERFORMED THERE.

By RICHARD P. STRONG.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

During the winter of 1910 to 1911, Manchuria was ravaged by an epidemic of pneumonic plague which in modern times knows no parallel. Upon the receipt of cable advices from the War Department, Washington, and the American National Red Cross Society, sufficient laboratory apparatus for emergency work in plague was hastily packed and Doctor Teague and the writer proceeded with this equipment by the quickest possible transportation to Mukden, Manchuria, arriving in this city on March first.[1] Here our services were at once placed at the disposal of the representatives of the Chinese Government.

The story of the Manchurian epidemic of pneumonic plague has been told at some length in the Report of the International Plague Conference, recently published in Manila under the supervision of the writer. In the present report it is merely the intention to recount our more important personal experiences and studies regarding pneumonic plague, either carried out by us in Manchuria or in this laboratory by ourselves or other members of the staff of the laboratory since our return. The epidemic had reached its height at Mukden a few days before our arrival there, and our investigations were immediately commenced at the plague hospital where we found about 50 cases of this disease on the occasion of our first visit. As is frequently

[1] The expenses of this expedition to China, where Doctor Teague and the writer acted as representatives of the American National Red Cross Society and as American representatives to the International Plague Conference, were largely paid for by the American National Red Cross Society and by the Chinese Government.

the case in other countries, when large epidemics of disease suddenly arise, China was unprepared to cope with this outbreak of plague. In Mukden there was no suitable building that could be used for a pneumonic-plague hospital. However, an old temple, situated about a mile from the city, had been converted for this purpose. (See Plate I.) About the various court yards of the temple numerous wards had been hastily constructed of light timber and boards, and the crevices between the boards covered with paper. Three small rooms, situated in the center of the hospital, were turned over to us for laboratory purposes. (See Plate II.) Tables, basins, etc., were supplied, and our laboratory apparatus having been installed, we began regular clinical and laboratory studies, which were continued until the end of the epidemic. In addition to the laboratory supplies brought with us, we ordered by cablegram from Messrs. Lautenschläger, Berlin, the emergency plague laboratory adopted by Professor Koch for the Institut für Infektionskrankheiten, Berlin, to be shipped to us by express. This laboratory apparatus is very compactly packed in five aluminum cases and cost, delivered in Mukden, exclusive of express, 6,925 marks. While with this additional equipment we were able to carry on satisfactory laboratory work, nevertheless, in our improvised laboratory building there was no running water, and even in the day time it was difficult to heat the rooms properly. At night, the temperature in the building was frequently below freezing point, so that incubators could not be kept at satisfactory temperatures. Obviously there was no gas in Mukden, and for bacteriological work alcohol blast lamps were employed and for sterilization purposes, primus burners. In addition, we found it difficult or impossible to replenish our chemicals and other supplies. These and other unfavorable conditions served as obstacles to the performance of ideal work, and many of our researches were, therefore, only completed in this laboratory after our return. We had previously purchased in Shanghai at the Municipal Laboratory, through the kindness of its director, Dr. Arthur Stanley, all the guinea pigs that that institution could spare. This proved a fortunate purchase, for we were unable to obtain any more of these animals during the entire time we were engaged in plague work in Mukden. Mice, however, were obtainable in limited numbers, and two species of marmots were kindly supplied us by the Chinese.

In the plague-hospital-wards, wooden platforms, about 70 centimeters high and 2 meters broad, and extending along one

wall the full length of the room, served as beds. (See Plate III.) On our first arrival at the hospital, we found that, owing to the great fear of contracting the disease that existed among the hospital attendants, the patients secured practically no medical attention. They were merely brought to the hospital to die, and the dead were removed each morning to the dead house. The patients lay on these platforms, or couches, side by side in their ordinary street clothes, and were not separated one from another in any way. We sometimes found patients with other diseases and with forms of lung trouble other than plague pneumonia on these couches, and in some instances we were able to save them from plague infection by the early diagnosis of the disease and by their speedy removal. The floors and walls of the wards were frequently spotted copiously with bloody sputum which had been expectorated upon them. We at once instituted a system by means of which an early diagnosis was made and an immediate bacteriological examination of the sputum of each case entering the hospital was performed. Later some beds were secured and many of the patients were placed on these. Also, hot tea and rice were supplied to them when they were able to receive such food. The wards were very inadequately heated by small iron stoves, and the temperature in them was at least during the night below the freezing point. The sputum in the sputum cups was frequently found in the morning to be frozen solid. No other European or American physicians attended the hospital, but there was a staff of Chinese doctors and medical students under the direction of Dr. Y. S. Wang, and towards the end of the epidemic an English male nurse was employed by the Chinese. In the early part of the epidemic a number of the native staff of the hospital became infected and died of plague.

We observed the strictest personal precautions against contracting the disease and never entered the wards unless fully protected by a proper mask, by goggles, usually rubber gloves, and by a cotton uniform. (See Plate IV.) Although we worked in the wards each day until the end of the epidemic and were often with patients for several hours continually, giving intravenous injections, leaning over coughing patients, exposing agar plates before them, making physical examinations, etc., we remained entirely healthy.

The type of mask which we used consisted of a cotton-wool pad, 12 centimeters wide, broadly folded in plain gauze, the two ends of which were each cut into three parts as a three-tailed bandage.

The tails of the bandage were tied one below the ear behind the neck, one above the ear, and the third above the head as a jaw bandage. The spaces on each side of the nostrils between the mask and the cheeks were plugged with cotton-wool. The whole mask was then covered with another piece of gauze in which openings for the eyes were made and the ends cut into four tails and tied behind the head and neck. (See Plate V.) This gauze served to keep the mask in place and to hold it securely against the face. While this form of mask appeared during the epidemic to be efficacious in preventing the wearer from contracting plague infection from a pneumonic-plague patient, two preliminary experiments performed in this laboratory by Teague, Barber, and the writer [2] have shown that this type of mask is not perfectly bacteria-proof. Since, however, as it has already been pointed out, we were frequently for hours at a time in close contact with coughing pneumonic-plague patients and remained entirely healthy, it appears that, during an epidemic, this form of mask is, at least, usually safe for practical purposes, and that while the gauze and cotton comprising the mask may not intercept bacteria, which are suspended and sprayed in a fine vapor in salt solution or even in saliva, they nevertheless usually intercept the fine droplets of sputum emitted, for example, by the cough of pneumonic-plague patients.[3] However, the very careful and complete experiments of Barber and Teague, for which they deserve entire credit,[4] throw considerable doubt upon the question of the degree of protection that would be afforded by this mask during a pneumonic-plague epidemic. These authors also demonstrate that the Broquet type of mask is more efficient.

[2] Report of the International Plague Conference held at Mukden, April, 1911. Manila (1912), 394.

[3] The droplets of mucus emitted from coughing pneumonic-plague patients are evidently much larger and heavier than the majority of those which are disseminated from an artificial spray of any kind and particularly is this true in the case of those where a force-pump is employed. Where the bacteria are suspended in saline solution and sprayed with a fine spray, the particles are always very much finer than those emitted from coughing individuals and have a much greater power of permeability. See Kirstein, *Ztschr. f. Hyg. u. Infectionskrankh.* (1900), 35, 123; Hutchinson, *ibid.* (1901), 36, 223. Laschtschenko states, *ibid.* (1899), 30, 132, the mucus droplets are also not so easily transportable as the particles from a spray. According to Heymann, *ibid.* (1899), 30, 139; (1901), 38, 21, the smallest droplets emitted by coughing tuberculous patients have a diameter of not less than 30 $\mu\mu$.

[4] See XII, p. 255 of this report.

Should an epidemic of pneumonic plague occur again, it is believed that it will be possible to employ in the wards female nurses for the care of the sick, with but moderate danger to themselves from contracting infection if they are properly protected with a suitable mask and uniform.

On our arrival in Mukden, in discussing the preliminary plans of our work, we were informed by some European missionary doctors that, owing to the sensitiveness of the Chinese people and to the reverence with which they regard the bodies of the dead, it would be quite impossible for us to perform necropsies in Manchuria, that necropsies had never been permitted, and if we should attempt to perform them, there would be riots among the people, and that we would surely be mobbed. Nothing of this kind was experienced, but frequently we found it difficult to secure necropsies. When we were first presented to the Viceroy of Manchuria, he asked for our assistance and advice in combating the plague. We replied that all assistance and advice possible would be given, but that at first it would be necessary to study the plague cases in the hospital, to have liberty to treat the plague patients, and to examine their bodies after death to see the effects of the treatment, etc. The Viceroy did not state definitely that permission would be granted us to perform necropsies, but spoke only of the danger from infection in performing them. However, he did not refuse to let us carry them on, and this was considered by us at the time as a satisfactory arrangement. In spite of the obstacles in obtaining pathological material from time to time, nevertheless we finally secured 25 perfectly fresh, complete necropsies.[5] We were told that these were the first post-mortem examinations that had ever been permitted in Mukden. (See Plate II, Necropsy room.) The examinations were sometimes performed under difficulties, owing to the extreme cold. The water in the buckets would sometimes freeze while the necropsy was being performed, and the blood formed icicles as it flowed upon and over the edges of the table.

The study of the pathological anatomy of the disease was considered important, owing to the fact that previously no such study had been pursued during an extensive epidemic of pneu-

[5] During the epidemic, Koulecha performed 28 necropsies in Harbin, the majority of which were upon bodies which were frozen and subsequently thawed, and Fujinami examined 26 bodies in Changchun and Dalny. See Report of the International Plague Conference, pp. 151 and 144.

monic plague. Tissues from each human necropsy, as well as many of the gross organs and pathological material from inoculated animals, cultures, microscopical specimens, etc., were brought by us to Manila and have been used in completing the work. It is believed that the study of this disease during the Manchurian epidemic and the further experiments relating to the subject, performed in this laboratory and outlined in the following pages of this report, have materially increased our knowledge of pneumonic plague.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

II. THE METHOD OF TRANSMISSION OF THE INFECTION IN PNEUMONIC PLAGUE AND MANNER OF SPREAD OF THE DISEASE DURING THE EPIDEMIC.

By RICHARD P. STRONG AND OSCAR TEAGUE.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Immediately after establishing our laboratory in Mukden, experiments were undertaken with the idea of elucidating the method of transmission of the infection in pneumonic plague and the manner of spread of the disease during the epidemic.

The sputum of pneumonic-plague patients in the advanced stages of the disease always contains enormous numbers of plague bacilli. The temperature of the hospital wards at Mukden was sufficiently low so that the expired air became immediately condensed to a vapor which was clearly visible to the eye as it issued from the mouth, and frequently could be seen for a distance of 30 centimeters or more from the face. In many of the patients advanced pulmonary œdema was present and the respirations were sometimes very forcible and sometimes even stertorous. Therefore, experiments were carried on to show, first, whether in cases of pneumonic plague the specific organism of this disease became disseminated into the air by the expired air or vapor arising from the breath in ordinary or dyspnœic respiration, and, secondly, whether this organism was disseminated by moderate attacks of coughing in pneumonic-plague cases in which the cough did not result in the expulsion of particles of sputum visible to the naked eye. These questions were studied extensively by means of exposing Petri dishes containing agar before undoubted plague cases and of then identifying the organisms which developed on the media by the usual bacteriological methods and particularly by animal inoculations.

In the course of the experiments, on a number of occasions during coughing, small droplets or larger particles of sputum, visible to the eye, were expelled, and touched the surface of the media in the Petri dishes which were exposed before the plague patient. The study of these cultures obviously is not included in this investigation. The Petri dishes containing agar were invariably exposed before cases of pneumonic plague with bloody sputum, in which enormous numbers of plague bacilli had been shown to be present. All of the cases before which the plates were exposed died of plague infection within twenty-four to forty-eight hours from the time of the exposure. Twelve series of experiments have been performed in which 82 plates containing agar were exposed and in 78 the microörganisms which have developed upon them studied as far as was practicable.

The experiments were performed in the following manner: The plates were sterilized in the hot-air sterilizer within a metal plate-holder. They were then removed, the agar-cultures melted and poured in the usual way, and, as soon as the medium was sufficiently hard, were replaced within the plate-holder and taken to the bedside of the patient in whose sputum plague bacilli had previously been found. All of the attendants were asked to retire from the ward in order that as little dust as possible might be present in the air. The condition of the patient before whom the plates were exposed was noted, and during the exposure of the plate the character of the respirations was particularly observed and notes made of whether coughing or talking occurred. The time of the exposure of the plate and the distance from the patient were also recorded in each instance. After the exposure, the plate was returned to the holder and placed in the incubator. Twenty-four hours later the plates containing the culture-media were examined for the appearance of colonies and the number of colonies counted, but the plates were not usually opened until after forty-eight or seventy-two hours. The colonies were then again counted and carefully studied. Any of the colonies which in any way resembled colonies of the plague bacillus were transplanted to slants of agar. The morphology and staining properties of the organisms on the plate- and agar-slant-cultures were then studied. In every instance in which the morphology was at all similar to that of the plague bacillus or the organism decolorized by Gram's stain, it was inoculated either into mice or guinea pigs. In a number of cases the colonies were so thick on the plate, or surface growths from contamination

with bacteria from the air were so extensive, that the separate organisms could not be isolated and studied. In a few of these instances a suspension of the whole growth upon the plate-culture was made, and a portion of the suspension either rubbed over the freshly-scarified abdomen of a guinea pig or inoculated subcutaneously into a mouse. On several occasions in which it seemed hopeless to determine whether the plague bacillus was present or not on the medium in the plate, owing to the extensive contamination of the culture with bacteria other than the plague bacillus, the guinea pig so inoculated died of plague. In some instances the plate-cultures were discarded because of very extensive contamination probably from air organisms which covered the whole surface of the medium with a very thick layer of growth. The ideal method would have been to inoculate guinea pigs by the cutaneous method with light scarification of the abdomen, with suspensions of the bacteriological growth on all those plate-cultures in which the separate colonies could not be isolated, and in this manner, perhaps, in others of these plate-cultures the presence of the plague bacillus might have been demonstrated. There is no more delicate a test for the presence of the plague bacillus than this procedure, and its efficacy is very great even when the few plague bacilli present are extensively overgrown by other microörganisms. Unfortunately, our supply of guinea pigs was limited to those we brought with us and none could be obtained in Mukden during the winter. Since we had numerous other experiments to perform, which also required the use of guinea pigs, we could only allow, while in Mukden, a very limited number for the present study. In the case of all of the organisms which suggested in any way the plague bacillus and the colonies of which had been transplanted to agar slants from the plates, inoculations of guinea pigs were made after our return to Manila.

During the colder weather in Mukden, the plates containing agar, exposed before plague patients during ordinary respiration, were frequently entirely sterile. The plates were usually exposed vertically before the mouth and nose of the patient, the time of exposure varying generally between two and five minutes; usually the shorter period was employed. In the experiments performed in the earlier part of the investigation, the plates were held at a distance of from 5 to 7 centimeters to 90 centimeters or 1 meter from the mouth of the patient. Later in the experiments, when it became evident that in cases without cough during exposure no plague bacilli were encountered at

the greater distances, they were exposed before cases which did not cough, usually at a distance of from 5 to 18 centimeters in front of the mouth and nose. Before coughing patients the distances varied from 5 centimeters to 2 meters. A summary of the details of the experiments follows.

EXPERIMENTS.

SERIES I.

On March 4, six plates containing agar were exposed in a ward containing 6 patients. Dimensions of ward about 3.5 by 4.5 meters. All plates exposed before pneumonic-plague cases, none of which coughed during the time of exposure.

Plates A and B exposed for three minutes at a distance of 30 centimeters from the mouth of the patient. Result after forty-eight hours: Both plates negative for colonies.

Plates C and D exposed for one and one-half minutes at a distance of 70 centimeters. Result after forty-eight hours: Both negative for colonies.

Plate E exposed for two minutes at a distance of 5 centimeters. Result after forty-eight hours: Three colonies developed on the plate. Two of these colonies are composed of a coarse bacillus which does not decolorize by Gram. The third is composed of a coccus.

Plate F exposed horizontally for eight minutes in the ward, 1 meter from the nearest patient. After forty-eight hours 8 colonies developed. On microscopical examination, none of the organisms composing these colonies resembled the plague bacillus, either in cultural characteristics, morphology, or in staining reactions.

SERIES II.

Case 1.—Pneumonic-plague patient; sputum not markedly blood-tinged, but patient very ill. Plate containing agar exposed at a distance of 5 centimeters from the mouth for one minute. Respirations quiet. Result after forty-eight hours: Two colonies have developed on the plate, one a large white colony of a coarse bacillus which does not decolorize by Gram's stain, the other a small and delicate colony planted on an agar slant. This organism partially decolorizes by Gram's stain. Mice Nos. 4 and 6 inoculated subcutaneously with 0.5 *æse* of this organism. Neither of these animals developed plague.

Case 2.—Sputum slightly bloody; respirations quiet. Plate exposed at a distance of 5 centimeters for one minute. Result after forty-eight hours: Negative for colonies.

Case 3.—Patient asleep. No coughing during exposure. Plate exposed for two minutes at a distance of 5 centimeters from the mouth. Result after forty-eight hours: Plate negative for colonies.

Case 4.—Patient very ill. Advanced case of plague pneumonia, with much bloody sputum. Vapor arising from breath. Marked dyspnœa. Groaned slightly while breathing.

Plate A exposed at a distance of 5 centimeters for one minute. Result after forty-eight hours: One colony, a pleomorphic bacillus, with square ends. Evidently not plague.

Plate B exposed at a distance of 5 centimeters for one minute. Result after forty-eight hours: Three colonies. All examined and proved to be a very large bacillus which does not decolorize by Gram.

Plate C exposed for two minutes at a distance of 5 centimeters. Result after forty-eight hours: Negative for colonies.

Case 5.—Patient delirious. Plate exposed for two minutes at a distance of 11 centimeters. Patient coughed slightly while plate was exposed. Result after forty-eight hours: Negative for colonies.

Case 6.—Advanced case with bloody sputum. Temperature 39° C.

Plate A exposed for forty seconds at a distance of 11 centimeters. Patient coughed slightly twice during exposure. No visible sputum on agar in plate. Result after forty-eight hours: A single large colony, resembling a colony of the colon bacillus. Microscopically, a very small bacillus which decolorizes by Gram. Planted on an agar slant. This organism was later inoculated in a dose of 0.5 *œse* subcutaneously into guinea pig No. 5475. The animal remained healthy.

Plate B exposed for two minutes at a distance of 11 centimeters. No coughing during exposure. Result after forty-eight hours: Three colonies; 2 large white colonies which consist of a coccus, the third of a bacillus that morphologically might be the plague bacillus. The colony is small and delicate. This organism decolorizes by Gram's stain. It was planted on an agar slant and later inoculated in a dose of 0.5 *œse* subcutaneously into guinea pig No. 5487, which remained healthy.

Case 7.—Plate exposed for a few seconds at a distance of 11 centimeters. Patient coughed three times during the interval. Apparently no sputum touched the medium in the plate. One colony after twenty-four hours. After forty-eight hours no further organisms developed. The single colony consists of a large coccus.

Case 8.—Plate exposed for one minute at a distance of 11 centimeters. Patient talked slightly while plate was exposed. Result after forty-eight hours: Negative for colonies.

Case 9.—With much bloody sputum. Plate A exposed for one minute at a distance of 11 centimeters. No coughing during exposure. Result after forty-eight hours: Fifteen colonies; rather heavy and white in appearance. None of these suggest plague. Microscopical examination of a number of them reveals a coarse bacillus which does not decolorize by Gram.

Plate B exposed for two minutes at a distance of 15 centimeters. No coughing. Result after forty-eight hours: A single colony=a large coccus.

Case 10.—Plate exposed for fifteen minutes in ward horizontally about 1 meter from a very sick patient, with much bloody sputum. No coughing during time of exposure. Result after forty-eight hours: Four colonies of moulds and 3 large colonies with yellow centers; evidently not those of the plague bacillus. Microscopically=a coccus.

Case 11.—Very sick case. Much bloody sputum. Plate exposed for one minute at a distance of 5 centimeters. Patient coughed slightly during exposure. Result after forty-eight hours: Negative for colonies.

SERIES III.

Case 1.—Plate exposed for one minute at a distance of 11 centimeters from the mouth. No coughing. Result after forty-eight hours: About 50 colonies have developed on the medium. Some of these look macroscopically

as though they might be those of the plague bacillus, but microscopically a number of them consist of a large bacillus which does not decolorize by Gram's stain.

Case 2.—Plate exposed for two minutes at a distance of 11 centimeters. No coughing. Result after forty-eight hours: One large colony of a coarse bacillus which does not decolorize. A group of several hundred pin-point-sized colonies; evidently not plague colonies. Microscopical examination shows a large bacillus in long chains.

Case 3.—Plate exposed at a distance of 90 centimeters from the mouth for fifteen minutes. No coughing. Result after forty-eight hours: About 20 scattered colonies and a patch of several thousand (?) colonies that do not look at all like colonies of the plague bacillus. A number of these colonies examined consist of a fine bacillus which does not decolorize by Gram's stain.

Case 4.—Plate exposed for one minute at a distance of 11 centimeters. No coughing. Result after forty-eight hours: Negative.

SERIES IV.

Case 1.—Advanced case with bloody sputum. Much vapor arising from the mouth.

Plate A exposed for two minutes at a distance of 11 centimeters. No coughing. Result after forty-eight hours: One large spreading colony, evidently not plague. Two large white colonies, evidently not plague. Two pin-head-sized colonies might possibly be plague colonies. On March 17, only 3 colonies are present on the plate that could possibly be plague. These were transplanted and studied. They all consist of a very short bacillus which partially decolorizes by Gram's stain. This organism was later inoculated by the cutaneous method into guinea pigs Nos. 5462 and 5477, which remained healthy.

Plate B exposed in similar manner to Plate A. No coughing. Result after forty-eight hours: One-half of the plate is overgrown by a surface growth; the other portion is free from colonies. Discarded.

Case 2.—With much bloody sputum. Vapor arising from the mouth during respiration.

Plate A exposed for two minutes at a distance of 11 centimeters. Patient coughed four times during exposure. Result after twenty-four hours: Almost the whole surface of the plate covered with a thick growth, which may be that of the Hay bacillus. In addition, there are 14 pin-head-sized colonies which may be seen beneath this growth. Plate discarded.

Plate B exposed for two minutes at a distance of 11 centimeters. Patient coughed three times during exposure. No visible sputum on plate. Result after forty-eight hours: Entire plate overgrown, except near the edge. Here are situated 5 small white colonies. Two of these are pin-point in size; the others are larger. The large colonies=a bacillus which does not entirely decolorize. The small colonies entirely decolorize by Gram's stain. One of these planted on an agar slant and later inoculated cutaneously into guinea pig No. 5486, which died of typical plague infection with early inguinal buboes, four days after inoculation. The whole plate-culture was suspended in a little saline solution, and several *œsen* of this suspension rubbed over the shaved and scarified abdomen of guinea pig No. 11. This animal died four days later. At necropsy there were typical inguinal buboes and a typical plague spleen.

Case 3.—Much bloody sputum. Patient asleep during exposure and groaning slightly. Plate exposed for two minutes at a distance of 5 centimeters. Result after forty-eight hours: Two large yellow colonies, evidently not plague. On March 17, one red colony had also developed on this plate, but no other colonies.

Case 4.—Advanced case with bloody sputum. Patient coughed several times during exposure. Plate exposed for two minutes at a distance of 11 centimeters. Result after forty-eight hours: The whole surface of plate covered with a thick layer of growth which has the odor of the Hay bacillus. Plate discarded.

Case 5.—Advanced case with bloody sputum. Patient snoring during exposure. Plate exposed for two minutes at a distance of 11 centimeters from the mouth. Result after forty-eight hours: 11 colonies; 3 resembling colonies of the colon bacillus, the others are more delicate and might be colonies of the plague bacillus. March 17, the majority of these colonies are of bacilli which do not decolorize by Gram's stain. Three colonies are found of a bacillus which does decolorize. These organisms were planted on agar slants and later inoculated into guinea pigs Nos. 12, 5471, and 5472. All of these guinea pigs remained healthy.

No. 6.—Plate exposed in plague ward for nine minutes about 1 meter from the nearest patient. Result after forty-eight hours: The whole surface covered with a whitish growth, beneath which are about 40 large white colonies which do not resemble plague colonies. Plate discarded.

No. 7.—Plate exposed in the ward, same as No. 6. Result after forty-eight hours: Growth over entire surface of the plate. Impossible to study individual colonies. Plate, therefore, discarded.

SERIES V.

Case 1.—Very sick, with much bloody sputum. In last stages of the disease. Much dyspnœa and pulmonary œdema and much vapor arising from the mouth.

Plate A. Patient coughed once during exposure of plate which lasted for one minute at a distance of 11 centimeters from the mouth. Result after forty-eight hours: About 60 colonies are scattered over one-half of the plate. Some of these look as though they might be plague colonies. Three of these, the organisms comprising which decolorized by Gram's stain and were bipolar when stained, were planted on agar slants and later inoculated by the cutaneous method into guinea pigs Nos. 13, 5474, and 5304, all of which died of typical plague infection.

Plate B exposed for two minutes at a distance of 11 centimeters from the mouth. Patient coughed slightly during the time of exposure. Result after seventy-two hours: Thirteen colonies on the plate. Six, which were large white colonies, could not be those of the plague bacillus. Seven of them might be colonies of the plague bacillus. A number of these colonies planted on agar slants as follows: 1 B 1=a short bacillus or coccus; Gram-positive. 1 B 2=a very fine coccus; Gram-positive. 1 B 3, no growth. 1 B 4=a very small coccus. 1 B 5 and 1 B 6=a bacillus which decolorizes by Gram's stain; inoculated cutaneously into guinea pigs Nos. 14 and 5309; both of these animals died of typical plague infection.

Case 2.—Much bloody sputum, pulmonary œdema, and marked dyspnœa. Much visible vapor arising from the mouth.

Plate A exposed for two minutes at a distance of 11 centimeters.

No cough. Result after twenty-four hours: No colonies. After forty-eight hours: Sixteen colonies made up of moulds and yellow colonies. Two small white colonies which possibly might be colonies of the plague bacillus planted on agar slants. One of these=a coccus, the other=a bacillus which decolorizes by Gram. Growth on agar develops a deep yellow pigment; evidently not plague.

Plate B exposed for two minutes at a distance of 5 centimeters. No coughing. Result after seventy-two hours: Four colonies; 1 very large yellow, 1 small yellow, and 2 small white colonies. The white colonies are planted on agar. They are composed of a bacillus which partially decolorizes by Gram's stain. These cultures were later inoculated into guinea pigs Nos. 5320, 5470, and 5473 by the cutaneous method, all of which remained healthy.

Case 3.—Much sputum; temperature 40° C. Plate exposed for two minutes at a distance of 11 centimeters from the mouth. No coughing. Result after forty-eight hours: Twenty-two colonies, none of which resemble colonies of the plague bacillus. In growth on agar slants, morphology, and staining reaction, the organisms comprising a number of these colonies all differ essentially from the plague bacillus.

Case 4.—Advanced case with marked dyspnœa and pulmonary œdema. Coughed slightly during exposure of plate. Plate exposed for two minutes at a distance of 15 centimeters from the mouth. Result after twenty-four hours: Eleven colonies have developed, 3 or 4 of which might possibly be colonies of the plague bacillus. After seventy-two hours about 40 colonies have developed. Only about 6 of these could possibly be colonies of the plague bacillus. These were planted on agar slants. Four of these organisms proved to be cocci and 2 bacilli, which partially decolorized by Gram's stain. Cultures of the 2 bacilli were later inoculated by the cutaneous method into guinea pigs Nos. 5312 and 5465, both of which remained healthy.

Case 5.—Advanced case with much bloody sputum.

Plate A exposed for two minutes at a distance of 11 centimeters. Patient talked slightly during time of exposure. Result after forty-eight hours: One large and 12 pin-point-sized colonies. Microscopical examination shows these to be composed of a bacillus that does not decolorize by Gram's stain. Growth on agar too delicate for plague.

No. 6.—Plate 1 exposed horizontally in a plague ward containing 3 cases of plague for ten minutes. Patient coughing about 1 meter away from the plate. Result after twenty-four hours: Six colonies and 1 small group of colonies. After forty-eight hours, the whole surface of plate overgrown with a heavy growth. Impossible to identify colonies. Plate, therefore, discarded.

No. 7.—Plate 1 exposed in same ward containing 3 persons with pneumonic plague. Plate exposed for twelve minutes at a distance of about 2 meters from advanced case of plague. Patient coughed a number of times during exposure. Head turned in direction of plate. Result after forty-eight hours: Fifteen colonies and a thick surface growth covering almost the entire surface of the plate. The whole of this plate was suspended in a little saline solution and several *æsen* of this suspension rubbed over the shaved and scarified abdomen of guinea pig No. 6. This animal died five days later of plague infection with typical inguinal buboes and plague spleen.

SERIES VI.

Case 19.—Advanced case, breathing heavily. Temperature 40° C., pulse 130. Much bloody sputum, containing large numbers of plague bacilli.

Plates A and B each exposed for two minutes at a distance of 11 centimeters. Patient breathing heavily during the exposure. Surface of media in plates wet by the breath. No coughing during time of exposure. Result after forty-eight hours: Plate A, 16 colonies; 1 very large colony with irregular borders about 1 centimeter in diameter; 6 heavy white colonies from 3 to 4 millimeters in diameter which do not resemble colonies of the plague bacillus in any way; 2 groups composed respectively of 4 and 5 pin-point-sized colonies were examined microscopically and transferred to agar slants.

Results of cultures from Plate A: 19 A 1=a bacillus mostly staining as rods, a few taking the bipolar stain; this organism decolorizes by Gram. 19 A 2=a similar bacillus to 19 A 1. 19 A 3=a large diplococcus. 19 A 4 and 5=a large coccus. 19 A 6 to 9=a bacillus similar to 19 A 1. Culture 19 A 1 inoculated into mouse No. 13; dose 0.5 *cæ* subcutaneously; animal remained healthy. Cultures 19 A 2, 6, and 9 inoculated by the cutaneous method respectively into guinea pigs Nos. 5463, 5480, and 5481. All of these animals remained healthy.

Result of Plate B after forty-eight hours: A single small pin-head-sized colony. Evidently the same bacillus as 19 A 1. This organism grows very delicately upon agar.

Case 20.—Advanced case with bloody sputum. Temperature 40°C., pulse 134. Enormous numbers of pest bacilli in sputum. Three plates exposed before this patient, each for two minutes at a distance of 15 centimeters as follows:

Plate A. Patient coughed slightly three times during exposure. Result after forty-eight hours: Seven colonies had developed; 1 large colony with uneven edges; 1 very large surface colony and another similar but slightly smaller one. None of these could be colonies of the plague bacillus. The remaining colonies are pin-head in size and were planted on agar-slant-cultures as follows: Cultures 20 A 1 and 4=a short bacillus which partially decolorizes by Gram's stain. These cultures were later inoculated by the cutaneous method into guinea pigs Nos. 5464 and 5484. Both of these animals remained healthy. Culture 20 A 2=a thick bacillus which does not decolorize by Gram's stain. Culture 20 A 3=a short bacillus which also does not decolorize.

Plate B. Patient coughed slightly once during exposure. Result after forty-eight hours: Four colonies developed as follows: Culture 20 B 1=a spore-bearing bacillus; culture could not be plague. Cultures 20 B 2 and 3 are very large, heavy, white colonies;=a large spore-bearing bacillus which does not decolorize by Gram. Culture 20 B 4=a plump bacillus which also does not decolorize.

Plate C. No coughing during exposure. Result after forty-eight hours: Four colonies, 3 large pin-head-sized and 1 pin-point-sized. All transferred to agar-slant-cultures. Results as follows: 20 C 1=a very small bacillus; does not decolorize by Gram. 20 C 2=a bipolar staining organism, but does not decolorize at all by Gram. 20 C 3=a coccus. Colony on agar, yellow. 20 C 4=a bacillus; colony white, rather heavy for plague; morphology does not resemble that of the plague bacillus. This organism was

inoculated later into guinea pig No. 5461 by the cutaneous method. The animal remained healthy. Culture 20 C 5 shows very heavy white colonies of a coarse bacillus; evidently not plague.

Case 21.—Advanced case with bloody sputum, containing enormous numbers of plague bacilli. Temperature 40° C., pulse 130. Marked dyspnœa, much vapor arising from the mouth.

Plate A exposed for two minutes at a distance of 11 centimeters. No coughing during exposure. Surface of plate wet by the vapor from the breath. Result after forty-eight hours: Fifty-eight colonies. Most of these do not resemble colonies of the plague bacillus. Nine of the colonies that might possibly be plague were inoculated on agar slants and studied further as follows: 21 A 1 and 2=cultures of bacilli that do not decolorize by Gram; morphology not right for plague. 21 A 3=a coccus or coccobacillus which decolorizes by Gram; 0.5 *œse* of this culture was inoculated subcutaneously into mouse No. 12 and 1 *œse* cutaneously into guinea pig No. 5482; both of these animals remained healthy. Culture 21 A 4=a bacillus which does not resemble the plague bacillus morphologically and which only partially decolorizes by Gram's stain; 1 *œse* of this culture was inoculated by the cutaneous method into guinea pig No. 5483; the animal remained healthy. Cultures 21 A 5 and 6=large bacilli, Gram-positive. 21 A 7=the same culture as 21 A 4. 21 A 8=the same bacillus as 21 A 3; inoculated into guinea pig No. 5322 by the cutaneous method; this animal remained healthy. 21 A 9=a bacillus whose morphology does not resemble the plague bacillus; only partially decolorizes by Gram's stain; 1 *œse* of this culture was inoculated by the cutaneous method into guinea pig No. 5485; this animal remained healthy.

Plate B exposed for two minutes at a distance of 11 centimeters. No coughing during exposure. Result after forty-eight hours: A large surface growth covers two-thirds of the plate, in which are situated 3 rather large white colonies which do not resemble plague. Three small isolated colonies, situated near the edge of the plate, might possibly be plague. Microscopically, these colonies are made up of a small bacillus which only partially decolorizes by Gram's stain. One of these colonies was transplanted to an agar slant and later inoculated into guinea pig No. 5479 by the cutaneous method; this animal remained healthy.

SERIES VII.

Case 25.—Advanced case with much bloody sputum, containing enormous numbers of plague bacilli. Patient died two hours after exposure was made. Plates exposed for two minutes at a distance of 15 centimeters from the mouth.

Plate A. Patient coughed four times during exposure. Result after twenty-four hours: Twelve colonies. After forty-eight hours, 33 colonies. All of the colonies, which looked at all suspicious of plague, planted on agar slants Nos. I to IX. A suspension was then made with 0.5 centimeter of peptone solution of all the colonies on the plate and 4 *œsen* of this suspension rubbed over the scarified abdomen of guinea pigs Nos. 8 and 15 respectively. Guinea pig No. 8 was found dead less than twenty-four hours after inoculation. There were no evidences of plague infection. Guinea pig No. 15 was found dead four days after inoculation. At the necropsy there were inguinal hæmorrhagic buboes and a typical plague spleen. Innumerable plague bacilli were present in the buboes and spleen.

The cultures formerly made from the colonies on the plate resulted as follows; 25 A 1=a coarse bacillus with square ends; decolorizes by Gram's stain; this culture was later inoculated into guinea pig No. 5307 by the cutaneous method; the animal remained healthy. Culture 25 A 2=a bacillus which decolorizes by Gram; growth on agar appears somewhat heavy for the plague bacillus; inoculated later into guinea pig No. 5459; this animal died of typical plague infection after five days. 25 A 3, 8, and 9=cultures of a spore-bearing bacillus; heavy white growth on agar. 25 A 4=a bacillus which partially decolorizes by Gram, but whose morphology is not right for plague and whose growth on agar is much heavier than that of the plague bacillus. 25 A 5 and 6=heavy white colonies; the same bacillus as 25 A 4. 25 A 7 decolorizes by Gram; morphology looks right; this culture was later inoculated into guinea pig No. 5460 which died six days later of typical plague infection.

Plate B. Patient talked a little during the time of the exposure and coughed once. Result after forty-eight hours: Sixteen colonies and a large surface growth. Only 3 of the colonies could possibly be colonies of the plague bacillus. These were planted on agar slants as follows: 25 B 1, colonies too heavy for plague;=a large spore-bearing bacillus. 25 B 2, a large bacillus; morphology not right, ends square, partially decolorizes; growth on agar not right for plague. 25 B 3=a bacillus which partially decolorizes; 1 *œse* of this culture was later inoculated into guinea pig No. 5308 by the cutaneous method; this animal remained healthy.

Plate C. No cough while exposed. Patient talked most of the time. Result after twenty-four hours: Eleven colonies. Result after forty-eight hours: Sixteen colonies. Only 4 of these colonies could possibly be those of the plague bacillus. These were planted on agar slants as follows: Culture 25 C 1, the colonies might be those of the plague bacillus, but are a little heavy. The organism is a bacillus which decolorizes by Gram; 0.5 *œse* inoculated subcutaneously into mice Nos. 18 and 26; both of these animals remained healthy; 1 *œse* of this same culture was later inoculated into guinea pig No. 5467 by the cutaneous method; the animal remained healthy. 25 C 2 and 3, the colonies have a yellowish-gray tinge; evidently not plague. 25 C 4, colonies have a deep yellowish-orange color; evidently could not be plague.

SERIES VIII.

Case 28.—With much bloody sputum, containing large numbers of plague bacilli. Temperature 38°C., pulse 132. Patient died twelve hours after exposure of plates. Physical signs in the lungs very slight.

Plate A exposed for two minutes at a distance of from 11 to 15 centimeters. Patient snored during the time of exposure. Result after forty-eight hours: The whole plate covered with a very heavy buff-colored surface growth. Plate, therefore, discarded.

Plate B exposed for two minutes at a distance of from 11 to 15 centimeters. Patient coughed slightly once during the time of exposure. Result after forty-eight hours: Sterile; no colonies have developed.

Plate C exposed for two minutes at a distance of from 11 to 15 centimeters. Patient coughed severely once. Result after forty-eight hours: About two hundred small colonies scattered over all parts of the plate, from pin-point to pin-head in size; one large light, buff-colored colony with irregular margins. A number of the small colonies planted upon

agar-slant-cultures. One-fourth of an *œse* made up of several of the small colonies on the plate was inoculated into mouse No. 16 and 0.25 *œse* of several of the other colonies into mouse No. 17, both subcutaneously; both of these animals died after forty-eight hours with marked swelling of the inguinal glands which contained innumerable plague bacilli; in each the spleen was swollen and contained innumerable plague bacilli; in each, cultures from the heart showed *Bacillus pestis*. Results of colonies transplanted previously from the plate to agar slants are as follows: Culture 28 C 1, a very short bacillus which resembles the pest bacillus morphologically and takes a bipolar stain; does not decolorize by Gram; 0.5 *œse* inoculated into mouse No. 21; the animal did not develop plague infection. Culture 28 C 2, short bacillus which does not decolorize by Gram's stain. 28 C 3, evidently the same organism as 28 C 1. 28 C 4, a coccus or very short bacillus which does not decolorize by Gram; evidently the same as 28 C 1. 28 C 5, colonies suggest those of *Bacillus pestis;* morphologically, a short bipolar staining organism; later inoculated into guinea pig No. 5457 by the cutaneous method; this animal died of typical plague infection six days after inoculation. 28 C 6, a coarse bacillus, evidently not plague. 28 C 7, evidently the same organism as 28 C 1, not plague. 28 C 8, 9, and 10, a bipolar organism which decolorizes by Gram's stain; 0.5 *œse* of 28 C 10 was later rubbed over the shaved abdomen of guinea pig No. 5458, which died of typical pest infection five days after inoculation.

Plate D. Patient coughed several times during exposure. Plate exposed at a distance of about 70 centimeters and only during the time of coughing. Result after forty-eight hours: A large surface growth covers about three-fourths of the plate. In this are situated about 50 colonies which might be plague colonies. Outside of this growth are situated 3 colonies which were planted on agar slants. Examined microscopically, the organism from these cultures is a very large bacillus which does not decolorize by Gram, but the culture does not look pure and in it there appear to be a few smaller bacilli which decolorize. For this reason, 1 *œse* of 28 D 1 and 1 *œse* of 28 D 2 were inoculated into guinea pigs Nos. 5454 and 5323 by the cutaneous method; both of these animals remained healthy; evidently the culture did not contain the plague bacillus.

Plate E exposed for one-half a minute at a distance of about 70 centimeters from the mouth of the patient. Patient coughed several times during exposure. Result after forty-eight hours: About 100 colonies are scattered through a large surface growth which covers the entire plate. Since it is impossible to isolate the colonies, the whole plate was suspended in a few drops of saline solution and several *œsen* rubbed over the scarified abdomen of guinea pig No. 9; this animal was found dead four days later. There were marked inguinal buboes on both sides and the spleen showed miliary abscesses; innumerable plague bacilli were present in smears from the spleen and buboes, and *Bacillus pestis* was isolated from the heart.

Plate F exposed for two minutes at a distance of about 5 centimeters. No coughing during exposure. Result after forty-eight hours: Eight colonies. Only 5 of these could possibly be plague. These were planted on agar slants. Only 3 of them looked at all like plague colonies. 28 F 1, 2, and 4 might be plague colonies; however, a microscopical examination

shows a very large bacillus which does not decolorize. Cultures 28 F 3 and 5 reveal a coarse bacillus which only partially decolorizes; these cultures were later inoculated into guinea pigs Nos. 5468 and 5469 by the cutaneous method; both of these animals remained healthy.

Plate G exposed at a distance of about 70 centimeters. Patient requested to cough, which he did 8 times. Plate exposed for a few seconds during the period of coughing. Result after forty-eight hours: About 100 colonies are scattered over the surface of the plate from pin-point to pin-head in size and up to a little larger in diameter. Thirteen of these colonies which resembled more or less colonies of the plague bacillus were planted on agar slants. One-fourth *œse* of several suspicious-looking colonies on the plate were inoculated subcutaneously into mouse No. 14, and another 0.25 *œse* of these colonies into mouse No. 15; one animal died forty-eight hours later and the other five days later of plague infection; in the first, the inguinal glands were swollen and contained innumerable plague bacilli, while in the second there was a typical left inguinal bubo; innumerable plague bacilli were present in the spleen of each animal. All the colonies on the plate (28 G) exposed before this patient were suspended in a few drops of saline solution and several *œsen* rubbed over the shaved and scarified abdomen of guinea pig No. 19; the animal died three days later with typical buboes and plague spleen; smears from the bubo and spleen showed innumerable bipolar organisms. The results of the agar-slant-cultures made previously from the colonies on the plate are as follows: Culture 28 G 1=a bacillus which does not decolorize. 28 G 2=a bipolar staining bacillus which decolorizes. Inoculated later into guinea pig No. 5455 by the cutaneous method; the animal died six days later of typical plague infection. 28 G 3 did not develop on agar. 28 G 4=a bipolar organism which decolorizes by Gram's stain. 28 G 5, a similar organism to 28 G 4; inoculated later into guinea pig No. 5456 by the cutaneous method; the animal died six days later of typical plague infection. 28 G 6=a bacillus which does not decolorize by Gram's stain 28 G 7, 8, and 9=a bipolar organism which decolorizes by Gram's stain; 28 G 9 inoculated later by the cutaneous method into guinea pig No. 5324, which died of typical plague infection five days after inoculation. 28 G 10, a very short bacillus which does not decolorize. 28 G 11, 12, and 13, a bipolar organism which decolorizes by Gram's stain; probably the plague bacillus.

28 H, ward plate, exposed in the center of the ward at about 2 meters from the nearest coughing patient. Time of exposure, four minutes. After forty-eight hours, only 2 isolated, large white colonies and a surface growth over about one-half of the plate had occurred. Plate discarded.

Plate 28 I, ward plate, exposed in the same manner, 2 meters from coughing patient, as Plate H for ten minutes. Light surface growth covers the entire plate. There are a few colonies situated beneath this. Plate discarded.

SERIES IX.

Case 29.—Three plates exposed for a few seconds each about a quarter of an hour before death of the patient, at a distance of about 11 centimeters from the mouth. Much vapor arising from the breath. No cough during time of exposure. Result after forty-eight hours: On all 3 plates, no colonies have developed; after seventy-two hours, plates still sterile.

SERIES X.

Case 32.—Advanced case, temperature 39°.2 C., pulse 110. Pulmonary œdema of both lungs; much bloody sputum, containing innumerable plague bacilli.

Plate A exposed for two minutes at a distance of 11 centimeters. No coughing during exposure. Talked slightly. Result after forty-eight hours: A surface growth covers the entire plate. It was, therefore, impossible to study any isolated colonies beneath this. The whole plate was, therefore, suspended in a few drops of peptone solution and mouse No. 23 inoculated with several *œsen* subcutaneously. This animal remained healthy.

Plate B exposed for two minutes at a distance of 11 centimeters. No coughing during time of exposure. Patient breathing quickly. Result after twenty-four hours: Plate sterile. After forty-eight hours, a heavy growth covers most of the plate, around the edge of which are a few delicate colonies measuring from 1 to 2 millimeters in diameter. Microscopical examination of these colonies shows a short bacillus which only partially decolorizes by Gram's stain; 2 *œsen* of a suspension of these colonies were inoculated subcutaneously into mouse No. 24, which did not develop plague infection.

Plate C. Patient coughed slightly about ten times during exposure. Result after forty-eight hours: Surface growth over four-fifths of the plate. A few small colonies in this growth. Four or 5 pin-point colonies outside the growth. Twenty-four hours later the whole plate covered by growth. Plate discarded.

Plate D. Exposed at a distance of about 70 centimeters. Patient gave one good cough during time of exposure which was for about one-fourth of a minute. Result after forty-eight hours: Surface growth over four-fifths of the plate. Three colonies outside of this which might be plague. Transplanted to agar slants. Results as follows: 32 D 1=a bacillus which decolorizes by Gram; morphologically, it looks rather thick for the plague bacillus, though from the colony, the organism might be the plague bacillus; inoculated later into guinea pig No. 5318 by the cutaneous method; the animal died seven days after inoculation with typical plague infection. 32 D 2=a bacillus which does not decolorize for the most part; however, there are a few organisms which are smaller and decolorize by Gram's stain; culture does not appear to be pure; 1 *œse* of this impure culture was later inoculated into guinea pig No. 5446 by the cutaneous method; the animal died of typical plague infection four days after inoculation; there were typical inguinal buboes and beginning miliary abscesses of the spleen.

Plate E exposed at a distance of about 30 centimeters for three-quarters of a minute. Patient gave one good cough during exposure. Result after forty-eight hours: Two irregular-shaped colonies that could not be plague; otherwise plate sterile. After seventy-two hours about 32 colonies, pin-point and pin-head in size scattered over the plate. Six of these colonies were planted on agar slants; microscopical examination of all revealed a bacillus which took a bipolar stain and decolorized completely by Gram's stain. Two of these agar-cultures were inoculated later by the cutaneous method into guinea pigs Nos. 5311 and 5447; the former died nine days after inoculation and the latter ten days after inoculation, both of typical plague infection with miliary abscesses in the spleen.

Plate F exposed for two minutes at a distance of about 70 centimeters,

while many attempts at coughing were made. Result after forty-eight hours: Large surface growth covering the entire center of the plate, in which are situated about one dozen smaller colonies. Impossible to isolate. Plate discarded.

Plate G exposed at a distance of 70 centimeters for a few seconds, while patient gave one good cough and made several attempts at coughing. Result after forty-eight hours: A number of suspicious-looking colonies present which take the bipolar stain and decolorize by Gram's stain. The organism is evidently the plague bacillus. Surface of plate became wet and plate, therefore, being dangerous to handle was disinfected.

Plate H exposed at a distance of 70 centimeters. One very good cough during time of exposure. Plate only exposed for a few seconds. Sputum was raised after the cough, but not during the cough. Result after forty-eight hours: Several hundred colonies which look suspicious for plague colonies. The colonies are not isolated, however, but in groups of very fine pin-point colonies and larger heavier colonies, about pin-head in size. A subsequent study of these cultures proves that 2 organisms are present on the plate; 1 a very small short bacillus which only partially decolorizes by Gram and whose colony is very delicate on agar and the other a bipolar-staining organism which entirely decolorizes by Gram. The latter organism was inoculated by the cutaneous method into guinea pigs Nos. 5310 and 5448, both of which died of typical plague infection, the former eight days after infection, the latter three days after infection. Two of the cultures of the organism which formed very delicate colonies on agar and which only partially decolorized by Gram's stain, were inoculated into guinea pigs Nos. 5313 and 5317, both of which remained healthy.

Plate I exposed at a distance of about 85 centimeters from the mouth for one and one-half minutes. Two good coughs during time of exposure. No visible sputum on plate. Result after forty-eight hours: Several hundred colonies that look suspicious of the plague bacillus. Colonies in small groups. Four of these groups planted on agar. Culture 32 I 1 did not develop; cultures 32 I 2, 3, and 4 all revealed a bipolar organism which decolorized by Gram's stain; three of these cultures were later inoculated into guinea pigs Nos. 5303, 5449, and 5450, all of which died of typical plague infection four days, six days, and eight days, respectively, after inoculation.

Plate J. Patient coughed during exposure. Small amount of sputum touched the plate. Plate, therefore, discarded.

SERIES XI.

Case 33.—Much bloody sputum which contained innumerable plague bacilli. Temperature 39°.8 C. Physical examination shows tubular modification of breath sound and signs of early œdema of the lungs.

Plate A exposed at a distance of about 85 centimeters. Patient gave five coughs during time of exposure. Plate then closed. Result after forty-eight hours: About 100 colonies scattered over the surface of the plate. Many look like plague. A number of these transferred to agar-slant-cultures. Subsequent study of these cultures shows that the majority consists of a bacillus which takes a bipolar stain and is completely decolorized by Gram's stain. Three of these cultures were later inoculated into guinea pigs Nos. 5302, 5451, and 5452 by the cutaneous method; all of these

animals died of typical plague infection; the first, four days after infection; the second, seven days after infection; and the third, eight days after infection. In addition to this organism on the plate, there was also present a small bacillus which has a very delicate colony and which usually does not decolorize by Gram's stain. This organism was encountered previously on other plates and is not the plague bacillus, as has been shown also by animal inoculation.

Plate B exposed at a distance of 85 centimeters, while the patient gave six good coughs. Result after forty-eight hours: A heavy surface growth in which several hundred colonies are situated; only 1 colony outside the edge of the surface growth. On microscopical examination, this proves to be a very short bacillus or coccus which does not decolorize by Gram's stain.

Plate C exposed at a distance of 85 centimeters for fifteen seconds, while the patient gave 4 coughs. Result after forty-eight hours: About 100 colonies scattered over the surface of the plate; many look like plague colonies. Five of the most suspicious were planted on agar slants; microscopical examination of all of these cultures shows a bipolar-staining bacillus which decolorizes by Gram's stain. Two of these cultures were later inoculated into guinea pigs Nos. 5301 and 5453 by the cutaneous method; both of these animals died of typical plague infection; one seven days after inoculation and the other nine days after inoculation.

Case 34.—Advanced case, partially delirious. Physical examination shows tubular respiration and signs of advanced œdema at the bases of the lung posteriorly.

Plate A exposed for two minutes at a distance of 11 centimeters from the mouth. No cough during time of exposure. Result after forty-eight hours: Many surface colonies. All colonies at all suspicious of plague were planted upon agar slants. A suspension was then made of all the colonies on the plate in a few drops of saline solution, and 3 *œsen* of this heavy suspension rubbed over the scarified abdomen of guinea pig No. 27; this animal remained entirely healthy. The result of the colonies previously transplanted on agar slants is as follows: 34 A 1 and 2=a bacillus, short and very thick which, however, decolorized completely by Gram's stain; this organism was later inoculated into guinea pig No. 5306, which remained healthy and did not develop plague infection. 34 A 3= a very large and thick bacillus which does not decolorize by Gram. 34 A 4=a coccus. 34 A 5=a bacillus which partially decolorizes by Gram's stain; this organism was later inoculated into guinea pig No. 5475, which remained healthy.

SERIES XII.

Case 35.—Advanced case of plague pneumonia. Patient with marked dyspnœa. Died about two hours afterward.

Plate A exposed for two minutes at a distance of 7 centimeters. No cough during exposure of plate. Result after forty-eight hours: About 28 colonies on the plate. Seven of these are large, irregular colonies which could not be plague. A number of smaller ones, from pin-point- to pin-head-size in diameter were planted upon agar slants. All the remaining colonies on the plate were then suspended in a few drops of peptone solution and 3 *œsen* of this suspension inoculated subcutaneously into mouse No. 25. This animal did not develop plague infection. The result of the colonies previously planted upon agar slants is as follows: 35 A 1

and 5=a bacillus with very delicate colonies which partially decolorizes by Gram's stain; these two cultures were later inoculated into guinea pig No. 5305, 1 *œse* of each culture being rubbed in different places over the shaved and scarified abdomen; the animal remained healthy. 35 A 2, 3, and 4 are cultures of a small bacillus which does not decolorize by Gram's stain; this culture was inoculated into guinea pig No. 5319, which remained healthy. 35 A 6=a bacillus which partially decolorizes by Gram's stain; this organism was later inoculated into guinea pig No. 5321, which remained healthy.

Plate B exposed at a distance of about 7 centimeters for two minutes. Patient breathing heavily; no cough during time of exposure. Result after forty-eight hours: Irregular surface growth, covering practically the whole surface of the agar in which many isolated, round colonies, from 2 to 4 millimeters in diameter, are situated. The growth on the plate was suspended in a few drops of peptone solution, and several *œsen* rubbed over the shaved and scarified abdomen of guinea pig No. 28 with a scalpel; the animal died six and one-half days after of typical plague infection; there was a hæmorrhagic local lesion about the point of inoculation; hæmorrhagic inguinal buboes; and the spleen showed early miliary abscesses; there was no pneumonia; smears from the spleen and bubo showed innumerable plague bacilli, and a culture of *Bacillus pestis* was obtained from the heart.

Plate C was exposed for two minutes at a distance of about 7 centimeters; no coughing during time of exposure. Result after forty-eight hours: Irregular surface growth covering almost entire surface of the plate, in which are scattered numerous colonies, 2 to 3 millimeters in diameter. Just outside the edge are 3 small white colonies, which are planted on agar slants. The growth on the surface of the plate was then suspended in a few drops of saline solution and 3 *œsen* inoculated subcutaneously into mouse No. 26, which did not develop plague infection. Results of the cultures on agar slants are as follows: 35 C 1=a bacillus which decolorizes by Gram's stain; the growth is rather heavy for plague; the culture was inoculated later into guinea pig No. 5478, which remained healthy. 35 C 2 and 3 are cultures of a large bacillus which partially decolorizes by Gram's stain; these 2 cultures were inoculated later into guinea pigs Nos. 5316 and 5315, which did not develop plague infection and remained healthy.

Case 36.—Advanced case of pneumonic plague. Plate exposed at a distance of about 85 centimeters for one and three-quarters minutes. Patient coughed five times during exposure. Only 3 colonies developed on the plate. These were transplanted to agar. Two of these colonies failed to develop; the third proved to be a coccus or very short bacillus which partially decolorized by Gram's stain. This organism was later inoculated into guinea pig No. 5314 by the cutaneous method. The animal remained healthy.

From these experiments it may be seen that of the 82 plates containing agar, 8 were exposed in the wards in the neighborhood of pneumonic-plague patients, 4 were exposed before patients who talked during the time of the exposure, and 35 before patients who coughed during the time of the exposure. In 39 instances the plates were exposed before patients who did not

cough during the time of exposure, and, notwithstanding the fact that many of the patients suffered with marked dyspnœa and advanced œdema of the lungs, in only a single instance was the plague bacillus encountered in one of these plate-cultures, although in a number of the experiments the surface of the medium was visibly wet by the vapor arising from the breath.

In this one case, the conditions of the experiment were as follows:

Three plates containing agar (Series XII) were all exposed at a distance of about 7 centimeters and for two minutes before a patient with marked dyspnœa and who died two hours afterward. A suspension of the bacterial growth upon one of these plates, which covered almost the entire surface of the plate, was made and a portion rubbed with the side of a scalpel over the abdomen of a shaved guinea pig and the skin then freshly scarified. The animal died of plague infection six and one-half days later; there were inguinal buboes and miliary nodules in the spleen. The animals inoculated with the colonies from the other 2 plates exposed in exactly the same manner did not develop plague infection. The results obtained from the examination of this one plate are different from those obtained from the remaining 38 plates exposed before patients who did not cough. Two possible explanations of the result suggest themselves, first, that the plague bacilli reached the medium on the plate exposed before the patient in the plague ward in some other way than by the expired air from the patient; and, secondly, that the plate was infected with plague bacilli by the droplet method through the forced expirations of the patient during the time this one plate was exposed.

The remaining number of plates (35)[1] were exposed before patients who coughed during the time of exposure, and in 15 instances colonies of plague bacilli developed on the media in the exposed plates. In some cases more than 100 colonies of this organism were obtained upon the media after a single cough, sometimes in almost pure culture.

Guinea pigs, the abdomens of which had been shaved and extensively scarified just before the time of the experiment, were exposed before 3 cases of pneumonic plague for a period of two minutes and at a distance of 5 centimeters from the mouth, the abdomen being placed toward the mouth. The breathing of the patients in all of these experiments was so

[1] In 4 other instances the patients talked during the time of the exposure, but no plague bacilli were demonstrated on these plates.

labored that the hair of the guinea pigs waved back and forth in the breeze made by the expired air, but no cough occurred during the time of the exposure. The animals remained alive, and did not develop plague infection.

The results of our experiments are in accord with the well-known bacteriological facts that bacteria are not detached from moist surfaces by ordinary currents of air, but that when sudden and forcible currents of air are forced from a distance through narrow apertures as, for example, from the trachea through the vocal cords, the tongue being against the gums and teeth, or through the lips, as occurs in talking or coughing, that small droplets of mucus, frequently invisible, may be emitted. The question of whether the expired air of patients afflicted with pulmonary tuberculosis was infectious was investigated particularly by Nägeli and Buchner [2] who demonstrated that such air was sterile. Flügge and his pupils, however, demonstrated that by coughing, tubercle bacilli were emitted in droplets from about 40 per cent of the tuberculous cases examined. Cornet and Meyer [3] after considering all of the experimental evidence concluded that droplet infection did not play an important rôle in the dissemination of tuberculosis.

In pneumonic plague the conditions are very different, owing to the enormous numbers of plague bacilli which are present in the lungs and bronchi. In our experiments, performed with cases of marked pulmonary œdema, the conditions were also different. The opportunities for infection by means of the droplet method must be very great in a pneumonic-plague ward. The distance from the patient that the air may be infected by droplets containing plague bacilli would apparently vary up to certain limits, particularly with the strength of the cough, the amount of mucus in the throat and larynx at the time, the size of the droplets emitted, the currents of the air in circulation, and the temperature [4] in the ward at the time.

CONCLUSIONS.

1. During normal and dyspnœic respiration of primary pneumonic-plague cases, plague bacilli are not usually expelled by means of the expired air.

2. During coughing of such cases, even when sputum visible

[2] Die niedern Pilze, München (1877), 53, 108. *Centralbl. f. d. med. Wiss.* (1882), 20, 513.

[3] Kolle und Wassermann, Handbuch der pathogenen Mikroorganismen (1903), 1, 146.

[4] See III, p. 157 of this report.

to the naked eye is not expelled, plague bacilli in large numbers may become disseminated into the air surrounding the patient.

The idea that infection of doctors, nurses, attendants, etc., in plague hospitals is caused entirely by particles of sputum expectorated by the patient and visible to the naked eye is erroneous. It follows from these experiments that the wearing of masks and the proper covering of any surface of the skin where fresh abrasions are present are important, personal, prophylactic measures against plague infection. It also follows that the eyes should be protected against this manner of conjunctival infection by proper glasses.

Articles of clothing worn in the wards should be sterilized immediately after removal, since plague bacilli may be present even though no particles of sputum may be visible upon them.

From these experiments, also, it is evident how dangerous an infective agent a pneumonic-plague patient is. In no other disease is the individual so dangerous and in no other disease does the danger from droplet infection approach that which exists in pneumonic plague. The number of plague bacilli expelled in droplets from pneumonic-plague cases is probably far greater than the number of bacilli ever expelled by patients afflicted with tuberculosis, croupous pneumonia, diphtheria, or influenza.

MANNER OF SPREAD OF THE DISEASE DURING THE EPIDEMIC.

During the epidemic the disease was evidently spread directly from man to man by droplet infection and by the more or less intimate contact of healthy individuals with an infected person. Whatever may have been the primary source of the epidemic, its dissemination occurred entirely independently of tarbagans, rats, donkeys, or any other animals.[5]

The disease was introduced into uninfected villages and towns by the importation of individuals infected with pneumonic plague or by those in the incubation period of this disease. No definite bacteriological evidence, that healthy carriers of the disease with plague bacilli in their sputa existed during the epidemic, has been produced. We had opportunity to examine two healthy individuals who were supposed to have given rise to the disease in other persons but who themselves remained healthy. We were unable to demonstrate any plague bacilli in their sputum, and it was not infective for guinea pigs.

[5] For evidence regarding dissemination by donkeys, see VIII, p. 225 of this report.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

III. INFLUENCE OF ATMOSPHERIC TEMPERATURE UPON THE SPREAD OF PNEUMONIC PLAGUE.

By OSCAR TEAGUE AND M. A. BARBER.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

In Manchuria, during the winter of 1910 to 1911, pneumonic plague spread with such rapidity that within three months 50,000 people died of the disease. Except toward the close of the epidemic, sanitary conditions were bad, the weather was bitterly cold, and quarantine measures were inadequately enforced. In India, where sanitary conditions are, perhaps, equally as bad, although there have been numerous isolated cases of pneumonic plague during the past fifteen years (from 2 to 5 per cent of all plague cases), this type of the disease has not assumed epidemic proportions.

Why was there a rapid spread of the pneumonic type of the disease in the one instance and a failure to spread in the other? The most obvious difference in the two instances is one of temperature, in the one case as low as 30° C. below zero as compared to 30° C. above zero in the other. Can the failure of pneumonic plague to spread in India be due to the high temperature that prevails in that country? If we consider only the direct action of the high temperature upon the plague bacilli, this question must be answered in the negative; for the optimum temperature for the cultivation of the plague bacilli upon artificial media is 30° C., which is approximately the temperature to which they would be subjected in India. We believe, however, that indirectly the temperature of the atmosphere is a factor of vast importance in the spread or failure to spread of pneumonic plague.

It is quite generally accepted that infection in pneumonic plague is due to the inhalation of plague bacilli and, as plague bacilli are readily killed by drying, it is fair to assume that infection is due to the inhalation of moist bacilli—the so-called "droplet infection." In plague pneumonia, the mucous membranes of the bronchi, trachea, larynx, and mouth are covered with enormous numbers of plague bacilli. It follows that such

a patient in coughing throws out droplets of sputum which must contain plague bacilli. Strong and Teague [1] demonstrated that this does in fact occur. Petri dishes, containing solidified agar-culture-medium, were held before the mouths of coughing plague patients, and, even when no visible particles of sputum appeared, colonies of plague bacilli developed on the plates. Granted that infection is due to the inhalation of droplets of sputum containing plague bacilli, it follows that the longer these droplets remain suspended in the air, the greater the danger of infection.

These droplets may disappear from the air in the immediate neighborhood of the patient in three ways; namely, (1) by evaporation, (2) by settling, and (3) by being borne away by currents of air.

The rate of evaporation depends chiefly upon the water deficit of the atmosphere. Under ordinary conditions this is far greater in warm weather than in cold and hence, ordinarily, evaporation of droplets of moisture in the air will take place far more rapidly in warm weather than in cold. At 4° C., with a maximum of moisture in the air, the water vapor has a pressure of only 6.0 millimeters of mercury; hence, even if the atmosphere were absolutely dry at this temperature, the water deficit would be small and evaporation would take place very slowly.

At 30° C., with a maximum of moisture in the air, the pressure of the water vapor amounts to 31.5 millimeters. With 70 per cent of moisture in the air, there would still be a greater water deficit (9.4 millimeters of mercury) than in a perfectly dry atmosphere at 4° C. In a cold climate, with snow on the ground and a rise of several degrees in temperature during the middle of the day, the water deficit of the air would be approximately zero during the greater part of the twenty-four hours. These were the conditions in Manchuria during the recent epidemic of pneumonic plague; hence there must have existed a very low water deficit in the air and little tendency for the droplets of sputum to disappear by evaporation. In India, on the contrary, with a temperature ranging around 30° C., there is usually a large water deficit in the air and hence the droplets of sputum would tend to disappear quickly by evaporation, thus leading to the death of the contained plague bacilli by drying.

According to curves given in the Report of the International Plague Conference, the temperature at Harbin during the course of the epidemic ranged between —9° C. and —32° C. and the humidity between 61 and 92. At —10° C., the vapor tension

[1] See II, p. 137 of this report.

of water is 2.09 millimeters of mercury and at —20° C., it is 0.92 millimeters. Hence, with an average humidity of about 80, the water deficit of the air at Harbin during the epidemic would be represented by from 0.4 to 0.2 millimeters of mercury. Under these circumstances, evaporation could take place only with extreme slowness.

In India, with a temperature of +30° and a humidity of 70, the water deficit of the air would be represented by 9.46 millimeters of mercury. In other words, evaporation would take place from twenty-five to fifty times more rapidly in India than in Harbin.

During the plague epidemics of both India and Manchuria, the fact that the poor people were much overcrowded in their living quarters undoubtedly hastened the spread of the disease. In Manchuria, on account of the bitterly cold weather, the doors and windows of the overcrowded houses were kept tightly closed. Under these circumstances, another factor is introduced of perhaps no small importance in its bearing upon the rate of disappearance by evaporation of droplets of sputum in the air; namely, the moisture in the expired air. In the cold, the moisture from the breath of the inmates of an overcrowded room would quickly saturate the air and reduce evaporation to a minimum, whereas the air of a similar warm room could take up large quantities of moisture without becoming saturated.

The following hypothetical case will illustrate the point in question. Let us assume that there are 10 men in a room 4 meters by 4 meters with the ceiling 4 meters high and that the room is without ventilation. If the air of the room had a humidity of 50 and a temperature of 30° C., it would become saturated after about four hours, for the room contains 64,000 liters of air. The expired air, which has a temperature of 37° and is saturated with moisture, totals about 4,800 liters per hour. The vapor pressure of air saturated at 37° is 46.7 millimeters of mercury, and of half-saturated air at 30° it is 15.7. Therefore, the men would have to breathe $\frac{15.7}{46.7}$ or approximately one-third of the air of the room in order to cause saturation of all of it. This would require $\frac{1}{3}$ of $\frac{64000}{4800}$ or about four hours.

If the air of the room had a humidity of 50 and a temperature of 8° C., the men would have to breathe $\frac{4.0}{46.7}$ or approximately one-twelfth of it in order to produce saturation, and this would require $\frac{1}{12}$ of $\frac{64000}{4800}$ or approximately one hour. Hence the air of the room at the lower temperature would become saturated in about one-fourth of the time required at the higher temperature.

Furthermore, the overcrowded rooms in a warm climate would in reality be thrown open and the moisture of the expired air would be consequently more or less rapidly dissipated, whereas in the cold climate conditions would approximate the hypothetical case at 8° C. just cited; hence the difference in the rate of evaporation of droplets in the air due to overcrowding in cold and in warm climates respectively would be, in fact, greater than is indicated by the figures in the hypothetical case just described.

The surface tension of water at 4° C. is 74.9, and at 30° C. it is 71.03. The surface tension being greater at the lower temperature, with the same amount of water deficit, evaporation would take place more slowly there than at the higher temperature. This is, therefore, an additional factor which would tend to cause droplets of pneumonic sputum to persist longer in the air in a cold climate than in a warm one. However, it is a factor of far less influence than the water deficit of the air and hence deserves no further discussion.

It seems highly probable that plague bacilli in suspended droplets of sputum would survive much longer at a low temperature than at a high one, even were the water deficit of the air the same in both cases; or, in other words, that with the same rate of drying, the bacilli would remain alive longer at low temperatures than at higher ones. This would, then, be also an important factor in causing pneumonic plague to spread more rapidly in cold climates than in warm ones.

It is noteworthy that the only large epidemic of pneumonic plague in India of which we have a record occurred during cold weather in Kashmir in the winter of 1903 to 1904. The epidemic is described by A. Mitra,[2] who stated that it lasted from November, 1903, to August, 1904, "but the virulence was only from December to March." "In the districts there were altogether 1,443 cases with 20 recoveries. The recoveries being bubonic cases, which were seen at the end of the epidemic." We judge from these statements that the epidemic of pneumonic plague lasted from December till March. Mitra says:

> The conditions of life in these villages during the month of January and February were extremely unfavorable. Everything round was frozen.

The Indian Weather Review shows that Srinagar, which was the center of the Kashmir epidemic, had, during the month of December, 1903, a mean daily temperature of 36°.1 F. and a

[2] *Indian Med. Gaz.* (1907), 42, 133.

mean humidity of 81°.0; during January, 1904, a mean daily temperature of 29°.1 F. and a mean humidity of 88°.0; during February, 1904, a mean daily temperature of 36°.0 F. and a mean humidity of 85°.0.[3]

Therefore the conditions were such that droplets of sputum suspended in the air would have had a tendency to evaporate to dryness only with extreme slowness.

Gill[4] appears to have been the only investigator who has devoted especial attention to the epidemiology of pneumonic plague in India. He says:

> Pneumonic plague presents well-marked features as regards its time of occurrence, which cannot be considered altogether accidental and without significance.
>
> In the four epidemics of which I have notes the time of its first appearance was as follows:
>
> | | | | |
> |---|---|---|---|
> | 1905–1906 | Epidemic | (Sept.–Sept.) | Jan. 24th, 1906. |
> | 1906–1907 | " | " | Feb. 1st, 1907. |
> | 1907–1908 | " | " | Dec. 13th, 1907. |
> | 1908–1909 | " | " | Oct. 10th, 1908. |
>
> The last outbreak in the 1907–1908 epidemic was on March 16th, and in the two former epidemics this was noted as about the time of the last outbreak and its occurrence after April 1st has not been noted.
>
> The characteristic of pneumonic plague is therefore its occurrence at the early part of the plague season, during the months of January, February, and March, that is, while the epidemic is on the increase but before it has reached its maximum intensity.
>
> Thus, while in the Punjab, the time of maximum intensity is April and the beginning of May pneumonic plague is chiefly prevalent in February.
>
> But not only is this the case, but it exhibits the same features in regard to its time of occurrence in the individual epidemics in villages.
>
> For as was exemplified in regard to the typical case of Mokal it was at the commencement of the epidemic that it appeared and it lasted a comparatively short time, being succeeded or replaced by a more prolonged bubonic outbreak.
>
> It is not easy to understand the reason for this, but it suggests that the organism of plague has acquired at this time an unusual or perhaps "exalted" degree of virulence which, however, it is not long able to maintain.
>
> I am unable to give any figures showing the actual prevalence of the disease or even to roughly estimate the proportion it bears to the general epidemic.
>
> Judging from reports one reads it is probable that it varies in different parts of India, and it is my impression that it is commoner in the comparatively cool climate of the Punjab than in the warmer and moister parts of India.

[3] These data are taken from observations made at 10 a. m. and 4 p. m. The 8 a. m. temperatures and humidity for the same months are: Dec., 1903, 28°.9 and 92°.0, respectively; Jan., 1904, 28°.4 and 93°.0; Feb., 1904, 33°.1 and 90°.0.

[4] *Indian Med. Gaz.* (1909), 44, 135.

That plague bacilli may be unable "to long maintain their unusual or perhaps exalted degree of virulence" by passage from lung to lung, as is suggested by Gill, appears to us to be highly improbable, since the experimental data at hand indicate that passage from lung to lung in susceptible animals is the method of choice and, perhaps, the only method of exalting the virulence of plague bacilli and maintaining the high virulence thus attained.

The epidemiological observations of Gill possess, however, great interest with regard to the influence of atmospheric temperature upon the spread of pneumonic plague. He found that pneumonic plague occurred during cold weather and ceased when the warm weather began, in spite of the fact that the number of bubonic cases was still on the increase. Unfortunately, he did not publish his notes in sufficient detail for us to determine the atmospheric temperature and humidity which existed during his several epidemics, but as far as his observations go, they indicate that the atmospheric temperature was probably a factor of importance in the spread of pneumonic plague and the suppression of the epidemic.

The only other epidemic of pneumonic plague of recent years of which we find a reliable record is the small one which occurred in Osaka, Japan, also in the cold season of the year. The first patient was taken sick on December 19, 1899. This case was quickly followed by twelve others, the last dying on January 13, 1900.

The above discussion has been confined entirely to pneumonic plague, but obviously the same ideas apply also to other pneumonias. In other pneumonias, however, it is not unlikely that the dosage and virulence of the inhaled bacilli and the susceptibility of the host at the time of exposure are factors of far greater importance than in plague pneumonia; hence, the influence of atmospheric temperature on their spread would be more or less obscured by these other factors.

We have endeavored to obtain experimental data confirmatory of the ideas advanced in the foregoing discussion. It was, of course, impracticable to perform actual experiments with plague bacilli sprayed into the air on account of the danger of contracting pneumonic plague. We, therefore, sprayed harmless bacteria and determined how they behave in the air under different conditions, believing that the results obtained would justify us in drawing conclusions as to how plague bacilli would act under similar conditions. We selected for most of the experiments *B. prodigiosus* and a yellow sarcina obtained from the

air. Those organisms possess the following advantages for these experiments: (1) They are harmless, (2) their colonies on agar are readily recognized on account of the characteristic pigment production, and (3) they differ considerably in their resistance to death by drying, the prodigiosus being killed more readily than the sarcina. In a few experiments the cholera vibrio was used; this organism is much more readily killed by drying than is *B. prodigiosus*. The following experiment demonstrates the relative resistance to death by drying of the three varieties of bacteria just mentioned and of the plague bacillus.

EXPERIMENT NO. 1.

Suspensions in 0.5 per cent sodium chloride solution were made from fresh cultures of sarcina, *B. prodigiosus*, plague, and cholera. The suspensions were passed through filter paper with the exception of that of the sarcina, which was filtered through cotton. Carefully cleaned slides were sterilized in the hot-air sterilizer and allowed to cool to room temperature. Pledgets of cotton were soaked in the suspensions, squeezed out thoroughly, and quickly rubbed over the surfaces of a series of the sterile slides. The slides were placed at intervals face down upon solidified agar in Petri dishes and brought into close contact with the agar by gentle pressure. After the first few minutes had elapsed, the remaining slides of the series were placed face down upon a sterile wire-netting frame in a box which was covered with a sheet of blotting paper. This was done to reduce the number of contaminating air-organisms upon the slides which were exposed for long intervals.

Each slide was left upon the agar for an hour or longer and then moved back and forth a few times over the surface and finally transferred to a second Petri dish. It remained in the second Petri dish overnight and was then removed. The number of colonies that developed on the first plate gave an indication of the number of bacteria that were alive on the slide, but the second plate merely furnished information as to whether or not living bacteria were present.

The result of one such experiment will be recorded in full.

Cholera suspension on slide.

(1,200,000,000 per cubic centimeter.)

Time exposed.	Plate No. 1.	Plate No. 2.
	Colonies.	
At once.	1,000	Positive.
Min.		
½	5	Positive.
1	0	Negative.
2	0	Negative.
3	0	Contaminated.
4	0	Negative.
6	0	Negative.
8	0	Negative.
10	0	Negative.

Plague suspension on slides.

(100,000,000 per cubic centimeter.)

Time exposed.	Plate No. 1.	Plate No. 2.
	Colonies.	
At once.	8,000	Positive.
Min.		
1	1,000	Positive.
2	100	Positive.
5	0	Negative.
10	0	Negative.
15	0	Negative.
21	0	Negative.
30	0	Negative.

Prodigiosus suspension on slides.

(273,000,000 per cubic centimeter.)

Time exposed.	Plate No. 1.	Plate No. 2.
	Colonies.	
At once.	1,000	Positive.
Hrs. min.		
0 15	6	Positive.
0 30	0	Positive.
0 45	0	0
1 00	1	0
1 30	0	0
2 00	0	0
2 30	0	0
3 00	0	0
3 30	0	0
4 00	0	0
4 30	0	0

Sarcina suspension on slides.

(3,000,000 per cubic centimeter.)

Time exposed.	Plate No. 1.	Plate No. 2.
	Colonies.	
At once.	1,000	Positive.
Hours.		
1	800	Positive.
2	400	Positive.
3	175	Positive.
4	17	Positive.
5	33	Positive.
6	0	1 colony.
20	0	Negative.

This experiment was done in a large room with the doors and windows closed. The bacteria upon the slides were exposed to diffuse daylight during the first few minutes and were then placed in a covered box. The temperature of the room ranged between 32°.5 and 33°.6 Centigrade and the dry-bulb thermometer registered about five degrees lower than the wet-bulb one.

This experiment indicates, as do several other similar ones that we have done, that the plague bacillus occupies an intermediate position between cholera and prodigiosus with regard to its resistance to death from drying. Sarcina is much more resistant than the other organisms.

Having determined the relative resistance to death by drying of sarcina, *B. prodigiosus*, and *cholera vibrio* when spread in a thin layer upon glass slides, we next planned an experiment to find the result with these same organisms when contained in fine droplets of saline solution suspended in the air.

EXPERIMENT NO. 2.

Fresh cultures of the bacteria were suspended in 0.5 per cent sodium chloride solution, the cholera suspension being made thicker than the prodigiosus and the latter thicker than the sarcina.[5] The spraying was done by means of an ordinary throat atomizer connected by rubber tubing with a double cylinder force-pump such as is used for filling automobile tires. The spray was directed during a period of half a minute toward all parts of a chemical hood, measuring 175 centimeters long, 80 centimeters deep, and about 2 meters high. Paper had been previously pasted over all cracks in the hood, and arrangements had been made for sliding a Petri dish into the hood over moist blotting paper by opening a small orifice for only two or three seconds.

The cholera suspension was sprayed first and plates were exposed in the hood at intervals until we judged (from preliminary experiments) that all the living cholera vibrios had disappeared from the air. The hood was then thrown open and about fifteen minutes later a similar experiment was performed with the prodigiosus suspension. Then the hood was again left open for a while and finally the experiment with sarcina was done. Each Petri dish containing solidified agar-culture-medium was left in the hood for a period of two minutes.

[5] The prodigiosus suspension was shown by plating out in agar to contain 120,000,000 organisms per cubic centimeter, the sarcina suspension, 33,000,000; the number per cubic centimeter in the cholera suspension was not determined.

Through the glass door of the hood, readings were made from the wet-bulb thermometer as follows:

Time.	Dry-bulb thermometer.	Wet-bulb thermometer.	Difference.
a. m.			°C.
9.37½ to 9.38. Cholera suspension sprayed.			
9.40	29°.9 C.	28°.2 C.	1.7
9.45	30°.2	28°.6	1.6
9.54	30°.6	28°.7	1.9
10.10½ to 10.11. Prodigiosus suspension sprayed.			
10.15	30°.0 C.	28°.2 C.	1.8
10.20	30°.4	28°.4	2.0
10.33	30°.65	28°.65	2.0
11.02	30°.8	28°.95	1.85
11.43½ to 11.44. Sarcina suspension sprayed.			
11.48	30°.3 C.	28°.6 C.	1.7
11.57	30°.85	28°.8	2.05
12.09	31°.05	29°.0	2.05
2.55	31°.8	30°.3	1.5

The results of this experiment are recorded in Table I.

TABLE I.—*Results of spraying suspensions.*

Time after spraying.	Cholera suspension.	Prodigiosus suspension.	Sarcina suspension.
Hrs. min.	*Colonies.*	*Colonies.*	*Colonies.*
0 ½	37,000	260,000	78,000
0 3	75	67,000	65,000
0 6	7	47,000	40,000
0 9	0	4,900	35,000
0 12	0	2,700	36,000
0 15	0	160	37,000
0 18	----------	25	28,000
0 21	----------	7	27,000
0 24	----------	7	25,000
0 30	----------	1	16,000
0 36	----------	1	----------
0 42	----------	0	----------
0 45	----------	----------	13,000
0 50	----------	0	----------
1 0	----------	0	10,000
1 15	----------	0	5,800
1 30	----------	----------	4,800
1 45	----------	----------	3,600
2 0	----------	----------	2,600
2 30	----------	----------	1,400
3 0	----------	----------	850

It is seen from the table that, when sprayed into the air under similar conditions, living cholera vibrios disappear from the air in about six minutes and living prodigiosus bacilli in about twenty minutes, whereas sarcina remains alive for more than three hours. There is a striking similarity shown by these organisms in their relative resistance to drying on glass slides and their persistance in the air when contained within fine droplets of saline solution. It would seem, therefore, that had plague bacilli been sprayed under similar conditions, the living ones would have disappeared from the air between six and twenty minutes after spraying.

This similarity in the behavior of the organisms on the slides and in droplets strongly suggests that also in the latter instance the disappearance of the living bacilli from the air is due to death from drying. If this were true, then if we were able to retard the evaporation of the water of the fine droplets, the living bacteria should remain in the air for a longer time. The most obvious method of retarding the evaporation of the fine droplets is to spray them into an atmosphere saturated with moisture. The following experiment was therefore carried out.

EXPERIMENT NO. 3.

The chemical hood used in the previous experiment was also employed for this one, but sheets of dry blotting paper were tacked against the walls and strips of cloth were tacked to the ceiling and allowed to hang down to within about 60 centimeters of the floor of the hood. A suspension of cholera vibrios in 0.5 per cent sodium chloride solution was sprayed into the dry hood, and Petri dishes were exposed for periods of two minutes each at intervals of three minutes until we could assume (from previous experiments) that living cholera vibrios were no longer present in the air. The entire interior of the hood was then thoroughly sprinkled with water and the cloths and sheets of blotting paper were also made soaking wet. After the wet hood had been kept tightly closed for some time, the same cholera suspension was sprayed into it for the same length of time as before; this time, however, the pump was placed in a tin vessel which was covered with towels soaked in hot water, so that the air going into the hood with the spray would contain more moisture. Plates were exposed as before for one hour. Then water was again sprinkled over the interior of the hood and a suspension of sarcina in 0.5 per cent sodium chloride solution was sprayed and followed a minute later by the same suspension of cholera that was used for the previous sprayings. Plates were exposed for three and one-half hours.

Temperature readings were made through the glass doors of the hood as follows:

Time.	Dry-bulb thermometer.	Wet-bulb thermometer.	Difference.
10. 51½ to 10.52.	Cholera suspension sprayed.		
10. 58 a. m.	30°. 6 C.	28°. 3 C.	2°. 3 C.
12. 02½ to 12.03.	Cholera suspension sprayed.		
12. 38 p. m.	31°. 0 C.	30°. 9 C.	0°. 1
1. 43 to 1.43½.	Sarcina suspension sprayed.		
1. 44½ to 1.45.	Cholera suspension sprayed.		
1. 50 p. m.	31°.95 C.	31°. 3 C.	0. 65
2. 00 p. m.	31°.31	31°. 26	0. 05

The results of this experiment are shown in Table II.

TABLE II.—*Results of spraying experiments.*

Time after spraying.		Cholera suspension in the dry hood.	Cholera suspension in the wet hood.	Wet hood.	
				Cholera.	Sarcina.
Hrs.	*min.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0	½	21,000	130,000	Innumerable.	104,000
0	3	170	33,000	34,000	31,000
0	6	0	24,000	4,700	15,000
0	9	0	2,600	1,800	13,000
0	12	0	470	195	7,000
0	15	0	220	300	6,000
0	18	0	38	20	3,000
0	21	.....	18	52	2,400
0	24	.....	8	18	2,800
0	27	.....	2	8	1,600
0	30	.....	0	4	1,700
0	36	.....	0	0	1,000
0	40	.....	0	0	900
0	50	.....	0	0	600
1	0	.....	0	0	360
1	30	.....	.....	0	135
2	0	.....	.....	0	86
2	30	.....	.....	0	31
3	0	.....	.....	0	16
3	30	.....	.....	0	1

In the dry hood the living cholera vibrios had all disappeared from the air six minutes after the spraying was discontinued, whereas in the wet hood living cholera vibrios were present after twenty-seven minutes. The wet- and dry-bulb thermom-

eters showed that the air of the wet hood was nearly saturated with moisture, and hence evaporation of suspended droplets of water must have been reduced almost to the minimum. Therefore, we are justified in concluding that the extremely rapid disappearance of the living cholera vibrios in the dry hood is due to the rapid evaporation of the suspended droplets of saline solution which leads to the death of the contained cholera vibrios from drying.

The last part of the experiment shows conclusively that the rapid disappearance of living cholera vibrios is not due to settling or removal through air currents, for droplets containing cholera vibrios and those containing sarcina were subjected to identical conditions and yet living sarcinæ were present in the air long after the cholera vibrios had disappeared. The sarcina being a larger organism and having a greater tendency to remain in clumps would settle out more rapidly than the cholera vibrio. It remained alive in the air longer than the cholera vibrios because of its greater resistance to drying. A similar experiment was performed with *B. prodigiosus*.

EXPERIMENT NO. 4.

A fresh culture was suspended in 0.5 saline solution and passed through filter paper. When plated out this suspension was found to have contained about 100,000,000 organisms per cubic centimeter.

The same suspension was sprayed for the same length of time into the dry hood, the wet hood, and into a cold storage room the temperature of which was about 18° C.

The temperature of air in the hood was read as usual through the glass door.

Time.	Dry-bulb thermometer.	Wet-bulb thermometer.	Difference.
a. m.			
9.47½ to 9.48. Prodigiosus suspension sprayed.			
9.55	30°.5 C.	29°.2 C.	1.3
10.12	31°.1	29°.0	2.1
11.02	31°.2	29°.25	1.95
p. m.			
12.05½ to 12.06. Prodigiosus suspension sprayed.			
12.20	31°.3 C.	31°.2 C.	0.1
12.50	31°.3	31°.25	0.05
1.50	31°.52	31°.42	0.1
2.30	31°.7	31°.6	0.1
3.40	31°.8	31°.7	0.1

In the cold storage room the spraying continued from 10.55½ to 10.56 a. m. The temperature of the room was as follows: At 11.02, 20°C.; at 11.30 a. m., 19°; at 12.35, 18°. Then it remained at 18° until the end of the experiment.

TABLE III.—*Showing growth of colonies.*

Time after spraying.	*B. prodigiosus* in dry hood.	*B. prodigiosus* in wet hood.	*B. prodigiosus* in cold room.
Hrs. min.	*Colonies.*	*Colonies.*	*Colonies.*
0 1	78,000	Innumerable.	44,000
0 5	52,000	Innumerable.	37,000
0 10	19,000	130,000	19,000
0 20	170	57,000	9,500
0 30	1	22,000	6,000
0 40	0	15,000	4,500
0 50	2	8,500	3,500
1 0	0	7,000	3,000
1 10	0	4,000	2,000
1 20	.....	1,500	2,000
1 30	.....	330	1,400
1 40	.....	170	1,300
1 50	.....	50	900
2 0	.....	15	750
2 15	.....	8	450
2 30	.....	.....	300
2 45	.....	0	350
3 0	.....	0	200
3 15	.....	.....	150
3 30	.....	0	150
3 45	.....	.....	125
4 0	.....	.....	90

As with the cholera vibrios so also in the case of *B. prodigiosus* there is a striking difference in the length of time that the bacilli remain alive in a dry and in a moist atmosphere. In the cold room the bacilli remain alive in the air even longer than in the wet hood. Unfortunately, the humidity of the cold room during this experiment was not determined.

It was, therefore, necessary to perform the following experiment. The same suspension of *B. prodigiosus* was sprayed for one-half a minute into a moist hood and into a cold storage room, and Petri dishes containing solidified agar were exposed in both places for periods of two minutes each at intervals of four hours. In the cold room the dry-bulb thermometer registered 12° C. and the wet-bulb one about 10°.5 C. throughout the experiment. In the hood the dry-bulb thermometer varied between 31.1 and 31.5 and the wet-bulb one registered about 0°.2 below the dry one. It is clear that the water deficit of the atmosphere was greater in the cold room than in the hood. The result of this experiment is recorded in Table IV.

TABLE IV.—*Showing growth of colonies.*

Time after spraying.	*B. prodigiosus* in moist hood.	*B. prodigiosus* in cold room.
Hrs. min.	*Colonies.*	*Colonies.*
0 1	Innumerable.	78,000
0 5	260,000	42,000
0 10	52,000	30,000
0 20	29,000	17,000
0 30	13,000	11,000
0 40	5,000	6,300
0 50	3,500	5,000
1 0	350	3,200
1 10	220	2,100
1 20	34	1,800
1 30	17	1,300
1 45	5	1,200
2 0	0	870
2 15	1	530
2 30	0	360
2 45	----------	330
3 0	0	320
3 15	----------	240
3 30	----------	160
3 45	----------	130
4 0	0	90

In spite of the fact that the water deficit of the air of the cold room was greater than that of the hood, the bacilli remained alive longer in the cold room. The only interpretation of this result is that *B. prodigiosus* resists death from drying longer at low temperatures than at high ones, even when the rate of drying is the same in both instances. It seems highly probable that this is also true of the plague bacillus; if so, the bearing of the phenomenon is an additional factor in the longer persistence of living plague bacilli in droplets of sputum, and hence upon the more rapid spread of pneumonic plague in cold climates is obvious.

SUMMARY.

It is shown that when spread on glass slides and exposed to the air, plague bacilli occupy an intermediate position between the cholera vibrio and *B. prodigiosus* with regard to resistance to death from drying. Sarcina resists much longer than *B. prodigiosus*. When suspended in saline solution and sprayed into the air, the living cholera vibrio disappears with surprising rapidity, *B. prodigiosus* persists for a longer time, and sarcina much longer than *B. prodigiosus*. The relative length of time that these organisms remain alive when sprayed into the air agrees strikingly with their survival on glass slides. This suggests that their disappearance from the air is also due to death from drying.

This was shown to be in fact the case by spraying the same cholera suspension into a comparatively dry atmosphere and then, under similar conditions, into an atmosphere nearly saturated with moisture; living cholera vibrios remained in the air much longer in the latter instance. A similar experiment was performed with *B. prodigiosus* with the same result.

By spraying sarcina and immediately thereafter cholera vibrios, so that the droplets containing these organisms were subjected to identical conditions, living sarcina was found to persist in the air long after the living cholera vibrios had disappeared. Since the sarcina is a larger organism than the cholera vibrio, it follows that the disappearance of the latter was not due to settling.

We believe we are justified in concluding from these experiments that were the plague organisms sprayed under similar conditions they would persist longer than cholera vibrios, but a shorter time than prodigiosus bacilli. Hence, it seems probable that the plague bacilli contained in fine droplets of pneumonic-plague sputum would suffer death from drying in a few minutes unless they were suspended in an atmosphere with an extremely small water deficit. Infection in pneumonic plague follows the inhalation of droplets of pneumonic sputum and obviously the longer these droplets remain suspended in the air, the greater is the danger of infection. As has just been stated, these fine droplets disappear very quickly except when they are suspended in an atmosphere with a very small water deficit. Such an atmosphere is under ordinary circumstances of common occurrence in very cold climates, whereas it is extremely rare in warm ones. Hence, since the droplets of sputum persist longer, the plague bacilli remain alive longer in the air, and there is a greater tendency for the disease to spread in cold climates than in warm ones.

In harmony with the above ideas, we find that the only great epidemic of pneumonic plague of modern times occurred in Manchuria during the winter of 1910 to 1911, when the atmospheric temperature was many degrees below zero Centigrade. The disease spread with amazing rapidity. Furthermore, although during the past fifteen years there have been millions of plague cases in India and 2 to 5 per cent of these have been cases of plague pneumonia, yet this form of the disease has not assumed epidemic proportions. The largest epidemic of pneumonic plague in India (1,400 deaths) occurred in Kashmir in northern India at an elevation of 1,524 meters above the sea level during very cold weather.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

IV. PORTAL OF ENTRY OF INFECTION AND METHOD OF DEVELOPMENT OF THE LESIONS IN PNEUMONIC AND PRIMARY SEPTICÆMIC PLAGUE: EXPERIMENTAL PATHOLOGY.

By RICHARD P. STRONG AND OSCAR TEAGUE.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

For the purpose of studying experimentally the question of the portal of entry of the organism and the method of the development of the lesions in pneumonic plague, animals were placed in closed glass cages, and agar-cultures of virulent pneumonic strains of the plague bacillus suspended in saline solution were sprayed for a period of from about two to three minutes into the surrounding air which they breathed. Thirty-four normal guinea pigs and 55 normal monkeys were so infected with plague bacilli, and all succumbed to plague infection. The animals were necropsied in each instance, and the lesions present observed and studied. It would be very tedious to record here the individual necropsy reports, since the lesions found were so often similar. Therefore, only a general description of the lesions will be undertaken, and the different types of lesions emphasized.

In the guinea pigs so infected, the following changes were encountered at necropsy. In general there was marked evidence of plague infection about the cervical and laryngeal tissues. The subcutaneous tissues showed extensive œdema, and there was swelling of the cervical lymphatic glands and of those about the trachea. Usually the glands were not only swollen but more or less hæmorrhagic and presented the appearance of small early buboes. Throughout the body marked evidences of septicæmia were usually present. There were frequently extensive hæmorrhages in the intestinal wall. The spleen sometimes showed the typical changes encountered in bubonic-plague in-

fection with miliary abscesses. Distinct evidences of pneumonia were present in only about 23 per cent of the guinea pigs. Plague bacilli were frequently not very abundant in the lungs, unless pneumonic areas were encountered, but were always present in the heart's blood. The lungs were sometimes reddened, congested, and œdematous, and sometimes contained hæmorrhagic infarcts. Small areas of primary bronchial pneumonia were encountered in some of the cases, and in one a whole lobe of the lung showed pneumonic engorgement. In two instances either red or early gray hepatization was present. Numerous miliary abscesses were occasionally encountered in the lungs. (See Plate VII.) The areas of bronchial pneumonia were firm, contained no air, and were usually irregular in outline and red, reddish yellow, or yellow in color. On cut section they were sometimes wedge-shaped. In those instances in which hæmorrhagic infarcts, miliary abscesses, and in addition reddish-yellow or yellow areas of lobular pneumonia are present (see Plate VII), we must conclude that the infection of the lung is secondary, and that in these instances we are not dealing with primary pneumonic plague, in which infection enters through the bronchi, but with secondary infection of hæmotogenous origin. Such a conclusion is supported by the microscopical study of these lesions. A section of the lung in the vicinity of one of the hepatized areas, pictured in Plate VII, shows the bacteria in very large numbers both about and within the small blood vessels, and in places infarctions have occurred; numerous hæmorrhages from the vessels have also taken place; in the neighborhood of the pneumonic areas the bacteria are also plentiful in the lung alveoli and in the perivascular spaces.

Therefore, these changes suggest that the primary point of infection did not always occur in the bronchi or alveoli of the lung. From a study of all the lesions in guinea pigs, it would appear that these animals, under the conditions of the experiments in which the spraying was carried on, did not frequently develop primary plague pneumonia, but that infection occurred through the mucous membranes of the mouth and throat, resulting in a general septicæmia generally preceded by the formation of early buboes of the cervical glands and sometimes followed by the development of secondary areas of plague pneumonia. It would appear that in guinea pigs, either on account of too shallow respiration or the small size of the larynx and trachea, the bacteria are not so likely to penetrate to the smaller bronchi by means of the inspired air. Instead, they are apparently

deposited largely upon the mucous membranes of the mouth and throat.

The experiments performed on monkeys seem to throw much more light upon the mode of pneumonic-plague infection in man. The lesions in 55 monkeys infected by spraying were studied at necropsy. There was a marked similarity in general in the pathological changes encountered. In practically all of the animals there was absence of any sign of plague infection about the cervical tissues. The submaxillary and cervical lymphatic glands and those about the trachea were not swollen, nor was there any œdema of the cervical tissues, as was practically always seen in the experiments with guinea pigs. In none of the cases examined did the tonsils show evidence of primary disease, though in a number of instances they were sectioned and stained. In some instances they were moderately congested. Plague bacilli were scanty in them and when present were not more numerous than in the heart's blood and never so numerous as they were in the lungs or spleen.

There was frequently œdematous fluid in the trachea, and in a few cases the trachea was slightly reddened. The larynx and vocal cords were not as a rule injected. There was not such marked evidence of septicæmia as seen in the experiments with guinea pigs, but plague bacilli could always be recovered from the heart's blood by culture. No hæmorrhages were noted in the intestines and omentum. The spleen and liver showed no miliary abscesses. There were no cervical, axillary, nor inguinal buboes. The lungs showed primary pneumonic changes in every case. There was always much œdema. In those animals which succumbed a shorter time after infection, the lobular type of pneumonia was much more frequently encountered. In those which survived a longer period, whole lobes of the lung usually showed pneumonia. The progress of the lesions is well shown in Plate VII, figs. 2 and 3. The process evidently begins as a lobular bronchial pneumonia. By the fusion of a number of the areas of lobular pneumonia, the whole lung may become involved. The large pneumonic areas were either in the stage of engorgement or of red or early gray hepatization. In a number of cases a pleuritic exudate was observed over the hepatized areas. In no case were miliary abscesses observed in the lungs. In the cases with the *early lesions*, the plague bacilli were always most numerous in the lungs, and in section were found in greatest profusion about the bronchioles, in the peribronchial lymph spaces and alveoli, and beneath the pleura. In some instances the cells

lining the alveoli appear normal even when they contain large numbers of bacilli. Although the blood vessels between the lobules and septa were dilated, and hæmorrhages sometimes occurred, practically no bacteria were found within them.

From these observations, it is obvious that the infection in monkeys occurred by inhalation and resulted in primary plague pneumonia.

It also is evident that in some instances in which monkeys are exposed to infection by inhalation, the primary point of infection may be not only the lungs, but also the mucous membranes of the mouth and throat. That plague infection may occur through the mucous membranes of the mouth and throat *alone* in monkeys was demonstrated by placing a small quantity of plague bacilli upon the posterior portion of the throat by means of a glass rod. The following experiments are given as examples of such infection.

EXPERIMENT I.

Monkeys Nos. 5882, 5883, and 5884 were all infected in the following manner on November 7.

A necropsy was performed upon monkey No. 5876 which had just died of experimental pneumonic plague and a portion of the pneumonic lung was cut into small pieces in a Petri dish. A glass rod with the end rounded in a flame was dipped into the œdematous fluid in the Petri dish and passed over the tongue and rubbed against the pharynx of each of the three monkeys (Nos. 5882, 5883, 5884). The monkeys held their tongues so that the glass rod was squeezed between the soft palate and the tongue and most of the material on the rod was evidently caught there. All three of the monkeys were treated in the same way and then the rod was dipped into the same fluid and touched to the shaved skin of a guinea pig as a control. The control guinea pig, No. 5885, died November 14, seven days after, with typical lesions of plague.

Monkey No. 5882 was found dead on Nov. 13, six days after infection. *Necropsy*: The superficial cervical glands are swollen on both sides. Both submaxillary glands are swollen and hæmorrhagic. The changes are more marked on the left side. The deep cervical glands on both sides are also swollen and hæmorrhagic; the process is more advanced on the left side. The axillary lymph nodes are also swollen and hæmorrhagic. The lesions in these glands are more marked on the right side. The tonsils on both sides are swollen and reddened. The larynx shows slight injection. The trachea contains a small amount of pale, frothy fluid; its mucosa is not injected. The bronchial lymph nodes at the bifurcation of the trachea are very small. There is no evidence of pneumonia in either lung. The spleen is much swollen and very soft. Smears from the spleen and blood show innumerable plague bacilli. Smears from the cervical and axillary glands show very numerous plague bacilli. Smears from the lung show fair numbers of plague bacilli. Sections of the tonsils show no evidence of primary plague infection and but few bacilli.

Monkey No. 5883 was found dead on November 15, eight days after infection. *Necropsy:* The tonsils are pale; they contain few pest bacilli and many cocci. The submaxillary, deep cervical, and axillary glands are small and deep red in color. A gland at the bifurcation of the bronchi is enlarged and reddish black in color and contains many plague bacilli. The pharynx and larynx are slightly reddened. The trachea contains some pale, frothy fluid. The lungs are pale and show no pneumonic areas. Cultures made. The spleen is enlarged, deep red, firm, and contains large numbers of plague bacilli. The blood also contains large numbers of plague bacilli.

Guinea pig No. 5902 inoculated with the spleen of monkey No. 5883. Died in four days with large numbers of plague bacilli in its spleen and with well-marked buboes.

Guinea pig No. 5901 inoculated with the lung of monkey numbered 5883. Died in seven days with large numbers of plague bacilli in its spleen and with well-developed buboes.

Monkey No. 5884 was found dead on November 14, seven days after infection. *Necropsy:* (By Dr. Crowell.) The tonsils and pharynx are considerably reddened and covered with frothy fluid. The tonsils are small and pale, show no hæmorrhages, and are probably not enlarged. The submaxillary glands are slightly enlarged and deep red. The deep cervical glands are somewhat enlarged and deep red, redder than the submaxillary. The glands in both axillæ are enlarged and hæmorrhagic, those in the left being larger than in the right. A gland at the bifurcation of the trachea is small, but deep red in color. The larynx and trachea are slightly reddened throughout their extent and contain abundant frothy, slightly blood-tinged fluid. The lungs show on the surface only a few small red areas. No pneumonia is present. On section, the cut surface is dark red and very moist. The spleen is enlarged and fairly firm.

EXPERIMENT II.

Culture No. 32 isolated from a pneumonic-plague case at Mukden was passed through a series of guinea pigs and a fresh culture from one of these passage guinea pigs was suspended in saline solution. A glass rod was dipped into this suspension and touched against the pharynx of three monkeys (Nos. 5927, 5928, 5929) as in the preceding experiment. The following necropsy reports show that the bacilli from artificial cultures brought about the same result as those inoculated directly from the pneumonic lung.

Monkey No. 5927.—Bacillus pestis placed on mucosa of mouth December 5. Found dead six days later. *Necropsy:* (By Dr. Crowell.) The axillary glands in the right axilla are slightly reddened, in the left they are pale. The tonsils show no visible change. There is no pneumonia present. The spleen is somewhat enlarged. Smears from the heart show numerous bipolar organisms. The spleen contains involution forms. The liver contains numerous bipolar organisms which are less numerous in the lungs than in the blood.

Monkey No. 5928.—Bacillus pestis placed on mucosa of mouth December 5. The animal died December 14. *Necropsy:* (By Dr. Crowell.) The tonsils, deep cervical, submaxillary, and axillary glands are only slightly swollen and reddened. The lungs are somewhat œdematous and a little

reddened. There is no consolidation. The trachea is only slightly reddened. The spleen slightly, if at all, enlarged. Smears from the heart's blood show very numerous pest bacilli, while the organisms in the lung, liver, and spleen are numerous.

Monkey No. 5929.—Inoculated on December 5, died on December 10. *Necropsy:* (By Dr. Crowell.) Smears from the spleen show very few, if any, pest bacilli. The heart shows two or three to a field. Few are found in the lung, while the liver shows numerous bacilli. The lesions are practically the same as those encountered in monkey No. 5928.

Therefore, these animals all died of plague septicæmia with or without bubonic infection of the cervical glands; that is, in the case in which the infection was severe and the susceptibility of the animals more marked, they succumbed to septicæmia before cervical buboes developed. In none of these instances was pneumonia present. Primary plague pneumonia only results when infection by inhalation has in addition taken place.

It has been claimed by several observers and more recently by Koulecha [1] that pneumonic plague in man is primarily a septicæmic disease, the lungs becoming secondarily involved by way of the blood circulation. According to this observer, the infection is supposed to spread from the perivascular spaces to the neighboring lung alveoli. He further believes that the bacilli enter the blood by the lymph vessels through the lesions in the tonsils and are deposited in the interstitial tissues around the lung alveoli, the tonsils being regarded as the primary point of infection. In some instances he assumes it to be possible for the plague bacilli to pass from the mucous membranes of the trachea and bronchi to the neighboring lymphatic glands and from them to enter the blood and in this way later to reach the lung. Albrecht and Ghon have shown that by the intravenous injection of plague bacilli in animals, pneumonic plague did not result.

In our opinion, the view that pneumonic plague is primarily a septicæmic disease and that the lungs become secondarily involved by way of the blood circulation and that the tonsil is first infected is not acceptable.

From our study of pneumonic plague both in man [2] and animals, we feel justified in concluding that infection in epidemic pneumonic plague results from inhalation, the primary point of

[1] Report of the International Plague Conference held at Mukden, April, 1911. Manila (1912), 154.

[2] For a description of the human lesions, see VII, p. 210 of this report.

infection being not in the tonsils[3] but some portion of the bronchi, the organism either passing along the bronchioles directly to the alveoli or through the walls of the bronchioles to the contiguous tissue of the lungs, giving rise, first, to peribronchial and perivascular inflammation in the surrounding tissues, and then to more diffuse inflammatory processes throughout the lung. Having reached the lung tissue, the bacilli rapidly multiply and produce at first pneumonic changes of the lobular type and shortly afterward more general lobar involvement of the lung tissue.[4]

The blood becomes quickly infected and a true bacteræmia results in every case. The fact that the bronchial glands at the bifurcation of the trachea are always much more severely affected than any of the other lymphatic glands also argues against the theory that epidemic pneumonic plague is primarily a septicæmic disease and that the lungs are infected secondarily from the blood. Moreover, in the earliest stage of the disease, the blood may be free from plague bacilli as we have shown by cultures.

It is true that in some instances the bacteræmia occurs early in the course of the disease and before hepatization of the lung may have taken place. However, microscopical examination will reveal enormous numbers of plague bacilli in the engorged lung tissue from which it appears that the origin of the bacteræmia is clear.

The tonsils may become secondarily infected in pneumonic plague just as other lymphatic glands—for example, the bronchial ones—become so infected. However, in pneumonic plague death usually occurs before any marked macroscopic changes occur in the tonsils. There is no doubt also that the tonsils may become primarily infected in epidemics of pneumonic plague just as has occurred in sporadic cases in epidemics of bubonic plague; such cases have been previously reported. This, however, is not the common channel of primary infection, and in such cases involvement of the glands of the neck occurs early in the course of the disease. Such cases are really instances of bubonic plague in which the lungs may, or may not, become secondarily infected.

In some instances plague infection may occur directly through

[3] See also under pathological anatomy, p. 215 of this report for the condition of the tonsils in the human cases and Plates XI and XVIII.

[4] See Plate VII, figs. 2 and 3, and Plates IX and X.

the mucous membranes of the mouth and throat. Primary septicæmia may then result. In those instances in which the infection is virulent and severe and the susceptibility of the host marked, death may sometimes occur before bubonic involvement is apparent. In other instances, bubonic involvement of the glands of the neck and septicæmia are present. No true pneumonia occurs unless infection by inhalation has in addition taken place. The German and the Austrian Plague Commissions concluded that primary plague septicæmia probably does not exist. However, these commissions made their observations only during epidemics of bubonic plague. From our studies made upon human beings, during the Manchurian epidemic, as well as from the animal experiments quoted above, we must conclude that primary plague septicæmia does sometimes take place and that death may occur, though rarely, before visible lesions have taken place either in the lungs or lymphatic glands.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

V. CLINICAL OBSERVATIONS.

By RICHARD P. STRONG AND OSCAR TEAGUE.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

CONTENTS.

TYPES OF THE DISEASE MET WITH DURING THE MANCHURIAN EPIDEMIC.

The cases throughout the epidemic were almost entirely those of primary pneumonic plague, only two or three undoubted cases of primary bubonic infection having been reported at the International Plague Conference.[1] However, in a number of instances death occurred before there were any clinical manifestations that pneumonic plague was present, and in some of these cases only at necropsy was it discovered that early involvement of the lungs existed. This led to the belief that many of the cases were primarily septicæmic in character. One observer at the Conference, Doctor Christie,[2] estimated from a clinical standpoint that about 5 per cent of the cases was of the septicæmic variety without pneumonia. However, from the post-mortem studies made during the epidemic, we must conclude that the cases with no involvement of the lungs were exceptional ones. Nevertheless, in a few instances in which infection did not occur by inhalation but probably through the tonsils or the mucous membranes of the mouth or throat, it seems unquestionable that the lungs were either not involved or only very slightly so. Thus,

[1] Report of the International Plague Conference held at Mukden, April, 1911. Manila (1912), 428.
[2] *Ibid.*, p. 166.

in one instance of this nature, occurring during the epidemic and reported by Fujinami,[3] the lymphatic glands of the neck showed enlargement with hæmorrhages, and the surrounding tissues of the pharynx and larynx were very much affected, while the lung was only very slightly involved. Obviously, this case should be regarded as primarily of bubonic character. Both the German and the Austrian plague commissions concluded that primary plague septicæmia probably does not exist. However, as has already been called attention to elsewhere in this report, these commissions made their observations only during epidemics of bubonic plague. From the studies made upon human beings during the Manchurian epidemic, as well as from the animal experiments performed in this laboratory,[4] we must conclude that primary plague septicæmia does sometimes occur, death resulting from this cause before lesions, which are macroscopically recognizable, are present in the lymphatic glands or in the lungs.

Several cases of primary intestinal plague were reported at the Conference in which bloody diarrhœa appeared to be the most prominent symptom. None of these cases was studied at necropsy. It appears that no definite evidence of the occurrence of primary intestinal infection during the epidemic was produced. In the few instances in which plague bacilli had been found during the epidemic in the fæces, infection had evidently occurred secondarily from the blood. Albrecht and Ghon in the report of the Austrian Commission have reported the only suggestive case of primary intestinal plague occurring during a bubonic epidemic of plague, and even in this case the evidence of such infection is not conclusive. However, it seems established that primary intestinal plague has been produced in rats by feeding large quantities of virulent plague bacilli. In many instances during the Manchurian epidemic, the patients with pneumonic plague must have swallowed enormous numbers of plague bacilli in the saliva and sputum. Nevertheless, in none of the necropsies performed during the epidemic were evidences of primary intestinal infection present nor was serious involvement of the intestine encountered. This fact certainly speaks strongly against the existence of primary intestinal plague in man and would seem to show that even if the intestines are sometimes secondarily involved, this condition in human beings must be also a very rare one.

[3] *Ibid.*, p. 150.

[4] See IV, p. 173 of this report.

SEX, AGE, AND INCUBATION PERIOD.

Both sexes seem equally susceptible, but the proportion of females and children attacked during the epidemic was comparatively small, as women and children were evidently not so frequently exposed to infection. The disease prevailed particularly among the poorer classes, coolies, etc., the majority of whom were between 20 and 40 years of age. The incubation period varied from two to five days, though usually it was not over two or three days.

SYMPTOMS.

The following summary of the clinical features of the disease has been made largely from personal observations during the epidemic in Mukden and also from evidence presented at the International Plague Conference.

The onset of the disease is usually somewhat abrupt; prodromal symptoms are rare. The disease usually begins with chilly sensations, but a distinct rigor generally does not occur. Epistaxis is generally not present. There is headache, loss of appetite, an increase in the pulse rate, and fever. Vomiting rarely occurs. Within from twenty-four to thirty-six hours after the onset, the temperature usually has reached 103° or 104° F., and the pulse 110 to 130 or more beats per minute. Cough and dyspnœa usually appear within twenty-four hours after the onset of the first symptoms. The cough is usually not painful. The expectoration is at first scanty, but soon becomes more abundant. The sputum at first consists of mucus which shortly becomes blood-tinged. Later the sputum becomes much thinner and of a bright-red color; it then contains enormous numbers of plague bacilli in almost pure culture. The typical rusty sputum of croupous pneumonia has not been observed. The conjunctivæ become injected, and the tongue coated with either a white or brownish layer. The expression is usually anxious, and the face frequently assumes a dusky hue. Labial herpes has never been observed. The patients sometimes complain of pain in the chest, but usually this is not severe. Apart from the disturbances due to the dyspnœa and their anxiety for their condition, they usually appear to suffer but little and usually do not complain of pain. In the later stages of the disease, the respirations become greatly increased and the dyspnœa usually very marked, the patients frequently gasping for air for several hours before death. Cyanosis is then common.

The signs of cardiac involvement are always marked in the

advanced cases, the pulse becoming gradually more rapid, feeble, and running; finally it can not be felt.

Gallop rhythm of the heart sounds are frequently observed. Death takes place from cardiac paralysis and exhaustion. The patients frequently succumb after slight physical exertion, such as sitting up in bed to take nourishment or on being moved. A few hours before death the temperature often declines to below normal. Delirium and coma are frequently present before death.

The urine in the later stages may show the presence of albumin. The diazo and indican reactions have not been observed in the few cases in which the urine was tested. Extravasations of blood have been found in the pelves of the kidneys at post-mortem examination.

The spleen is usually not palpable, and the lymphatic glands not enlarged. Petechiæ or larger hæmorrhages of the skin are usually not present. Bloodly diarrhœa is occasionally observed. Plague bacilli frequently may be present in the blood in such numbers that a simple, microscopical examination suffices for their detection; in other cases, cultures are necessary for their discovery. A marked leucocytosis may occur, though in some cases the leucocytes are not increased. In the *primary septicæmic* cases the course of the disease is very rapid. There may be no manifestations of disturbances of the lung. The cardiac symptoms are very prominent. The patients soon pass into a comatose condition and die.

PHYSICAL SIGNS.

The physical signs in the lungs are often slight, even in cases well advanced in the disease. On percussion, dulness is often absent, and the vocal fremitus and resonance unchanged. In a small proportion of cases, however, smaller or larger areas of dulness may be discovered. On auscultation râles are frequently not present, except shortly before death. When present early in the disease, they are usually of the fine variety. Numerous moist râles are heard late in the disease, and are due to the œdematous condition of the lungs. The character of the râles is in accordance with what one would expect from the condition of the lungs and bronchi and the character of the exudate observed at necropsy. Coarse râles such as occur in cases of catarrhal bronchitis usually are not present. Feeble, respiratory sounds, tubular modification, or pure tubular respiration over small areas are the conditions found most commonly on auscultation. Not infrequently a dry, pleuritic rub is heard.

The limits of dulness of the heart are sometimes increased

to the right of the sternum. The heart sounds are rapid and usually become feeble or embryocardiac in character toward the end. In the early stages the secondary pulmonic sound may be accentuated, but it soon becomes much less distinct.

DIAGNOSIS.

The diagnosis is usually clear from the bacteriological examination of the sputum in which the bacillus is found in enormous numbers and in almost pure culture. A rise in temperature and an increased pulse rate are usually the earliest symptoms observable, but before the sputum appears the diagnosis may be doubtful. An examination of the blood, either microscopically or by culture, may reveal the diagnosis, since during the past epidemic all the cases became septicæmic. The blood should always be examined early, by cultural methods, as in the primary septicæmic cases involvement of the lungs may not occur. The bacteriological diagnosis is the only certain one for excluding pneumonic infection due to microörganisms other than *Bacillus pestis*, but from the general condition of the patient, in connection with the absence of marked physical signs in the lungs, the diagnosis of pneumonic-plague infection is often particularly suggested. Labial herpes has not been observed in primary pneumonic plague. The presence of numerous coarse, piping or sibilant bronchial râles in the lungs is an argument against pneumonic-plague infection. The sputum in pneumonic plague is not purulent as it frequently is in catarrhal bronchitis or in bronchial pneumonia, and it is not so tenacious and has not the rusty appearance of the sputum so often seen in croupous pneumonia. The cough is usually not so painful as in croupous pneumonia.

The duration of the disease is usually less than two days, though many cases did not live longer than sixteen hours after the onset of symptoms. Cases sometimes survived for three, and, more rarely, for four days. In no case reported was the duration over one week.

PROGNOSIS AND TREATMENT.

The prognosis is unfavorable. No cases in which the bacteriological diagnosis was complete were known to have recovered during the Manchurian epidemic.

No method of treatment appeared in any way to have been successful. Treatment with serum seemed, in a few instances, to have prolonged the duration of the illness.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

VI. BACTERIOLOGY.

By RICHARD P. STRONG AND OSCAR TEAGUE.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

CONTENTS.

CHARACTERS OF THE PNEUMONIC STRAIN OF "BACILLUS PESTIS."

During the epidemic in Manchuria the idea became rather general that the organism of pneumonic plague differed, in some respects at least, from *Bacillus pestis* of bubonic plague. Apart from cultural variations, some physicians believed that while the bacillus of bubonic plague on inoculation into guinea pigs gave rise to buboes, the bacillus of this epidemic, on injection into these animals, caused only pneumonia and septicæmia. Also, it was claimed by some, that the virulence of the organism of pneumonic plague was much greater than that of the bacillus of bubonic plague. These ideas were erroneous, as is apparent from a consideration in detail of the properties of the pneumonic strain arrived at from the study of numerous microscopical preparations and cultures obtained from the sputum and from necropsies performed during the epidemic.

MORPHOLOGY.

From a morphological standpoint, the causative organism of the Manchurian epidemic of pneumonic plague apparently differs in no respect from other strains of *Bacillus pestis* isolated during

tremely difficult to break up the small clumps and to obtain a homogeneous and durable suspension. When grown at 37° C., the bacilli, when collected upon the platinum loop, form a homogeneous, moist, mucoid mass which readily forms a homogeneous suspension when shaken in saline solution. At 30° C., the growth results sometimes more like the growth at 37° C. just described, at other times more like the growth at the temperature of the ice-box, depending upon the strain employed; most strains cultivated at 30° C. produce more mucus than is usually seen in cultures developed in the ice-box and less than is seen in cultures grown at 37° C.

Another factor, which in our experience has exerted an important influence upon the mucus production of a plague strain, is the length of time it has been cultivated upon artificial media. Freshly-isolated strains, whether from human subjects or from our experimental animals, produce more mucus than strains which have been cultivated on agar for some time.

We cultivated a number of strains at 32° C. upon sugar-free agar, glucose-agar, saccharose-agar, and starch-agar, and did not observe that these carbohydrates caused an increase in the mucus production.

The age of the culture is a factor influencing the amount of mucus present. A twenty-four-hour culture will contain less mucus than the same culture several days later.

We have pointed out that the readiness with which the strains form homogeneous suspensions appears to run parallel with their mucus production and hence the former serves as a good index of the latter. There are, however, other factors which bring about the formation of homogeneous suspensions, notably the presence of alkali. The addition of a few drops of alkali to a suspension of a culture grown at ice-box temperature and shaking quickly brings about the disappearance of the clumps, and a homogeneous suspension results. It is for this reason difficult to determine whether or not the reaction of the culture-medium exerts an influence upon the mucus-production of plague organisms; we can only affirm that this influence, if present, is not marked.

To sum up, in our opinion the two factors of paramount importance with regard to the mucus production of plague bacilli are the temperature at which the cultures are grown and the length of time that the organisms have been cultivated on artificial media since their isolation from the animal host.

Bearing these facts in mind, we have not observed with regard

to mucus production that our pneumonic-plague strains in any way differ from the bubonic strains.

VIRULENCE.

The organism seems to have retained a maximum virulence throughout the epidemic, at least all of the cultures isolated and studied by inoculation into animals possess this very high degree of virulence. Cultures isolated near the close of the epidemic showed an equally high virulence to those isolated near its beginning. However, the idea that this epidemic of pneumonic plague was due to the fact that the strain possessed an abnormally high virulence—much greater than that possessed by the organism of bubonic plague—and that this accounted for the very high mortality during the epidemic appears to be erroneous. The very acute course of the disease, the very high death rate during the epidemic as compared with that of bubonic plague, and the apparently increased virulence of this pneumonic strain may be satisfactorily explained by the fact that the portal of entry of the organism and the location of the primary points of infection in pneumonic plague and in bubonic plague are different. The plague organism finds in the pulmonary tissues a much more favorable and extensive medium for its multiplication and diffusion than it does in the lymphatic glands. In bubonic plague, the lymphatic glands may be said to act as filters against the general invasion of the organism by the plague bacillus, while in primary pneumonic plague there is no such mechanism for the defense of the host, the bacilli spreading rapidly throughout the lung and invading the circulation in every instance in a comparatively short time and apparently before the organism has had time to produce any appreciable quantity of immune substances. The bronchial lymphatic glands in primary pneumonic plague offer resistance to the invasion of the plague bacillus, and in every case of this disease these glands are very acutely inflamed and frequently almost of a black color from the resulting toxic hæmorrhages in the glandular substance. However, by the time the bronchial glands have become involved, the bacteria have already spread so extensively throughout the lung substance that a bacteræmia has usually occurred. Microscopical preparations made at necropsy from the lungs of these pneumonic cases invariably contain enormous numbers of plague bacilli. In no other disease are the organisms found in such great abundance. In primary pneumonic plague, the bacilli are found in very much greater number in the lung than in the spleen,

even though an advanced bacteræmia is present. This fact, also, suggests that the lung tissue offers a more favorable location for the growth and multiplication of the bacilli than does the spleen. The bacteria are also present in far greater numbers in the lung than they are ever found in the buboes or spleen in bubonic-plague cases. It is, also, evident that in pneumonic plague the infected lung (which may be said to correspond to the primary bubo of bubonic plague) contains, by reason of the size of the infected area, a far greater number of plague bacilli than the primary bubo in bubonic plague. During epidemics of bubonic plague, there are occasionally small epidemics of pneumonic plague in which the same high mortality and acute course of the disease is observed as occurred in the Manchurian epidemic of pneumonic plague. This is another argument in favor of the fact that during epidemics of bubonic plague the causative organism may show the same high virulence. As examples may be cited the epidemic of bubonic plague in Japan—in Kobe and in Osaka in 1899 to 1900—in which 13 cases of primary pest pneumonia all terminated fatally after a very rapid course, and the epidemic of bubonic plague in 1898 in Bombay in which, toward its close, 11 cases of pneumonic plague also all quickly succumbed one after the other.

All this evidence is in favor of the supposition that the organism giving rise to the present epidemic is of no greater virulence than in the case of many bubonic strains; furthermore, definite proof of this fact has been obtained from comparative inoculations made in animals with different pneumonic and bubonic cultures. Many of our experiments have been reported in the testimony of the Conference, and will not be given in detail here;[1] the results of others performed by us since that time are recorded in Table I.

The guinea pigs were all inoculated in the following manner: An area of the abdomen, about 2 centimeters square, was shaved and scraped with the razor until petechial hæmorrhages appeared in the skin. A 42-hour agar-slant-culture of each organism was suspended in 5 cubic centimeters of peptone solution and 5 *œsen* of each suspension were rubbed over the shaved area of the guinea pig's abdomen. At necropsy the animals showed the usual lesions of bubonic plague. These guinea pigs were inoculated on June 8. The pneumonic strains were isolated during the month of March, the bubonic strain "Hongkong" on May 20, and the bubonic strain "Mariveles" on May 27. The bubonic strain sent from Shanghai had been on artificial media at least for several months.

[1] Report of the International Plague Conference held at Mukden, April, 1911. Manila (1912), 75, and Index under Virulence.

TABLE I.—*Showing virulence of various cultures of plague.*

Number of guinea pig.	Weight of guinea pig.	Number of culture.	Number of days before death.
	Grams.		*Days.*
5258	400	1	3½
5259	390	2	4
5261	350	5	6¼
5262	360	7	7½
5263	340	8	7
5264	400	9	3
5265	360	10	7
5266	350	21	6
5267	390	22	5½
5292	350	16	8
5293	380	23	6½
5294	390	25	6
5295	390	26	5
5297	330	28	3
5298	350	29	5½
5300	370	31	5½
5325	330	11	5
5326	350	12	7
5327	380	13	5½
5328	360	14	10
5329	380	15	5½
5330	360	17	5
5331	380	18	4½
5332	370	19	5
5334	350	Shanghai.	(a)
5335	360	Mariveles.	5½
5336	340	Hongkong.	5
5415	340	32	9

[a] Developed buboes, but recovered.

These experiments and those already referred to (*loc. cit.*) have shown that the pneumonic cultures have not possessed any greater virulence than that possessed by many virulent bubonic ones of the organism. Mice, rats, guinea pigs, and monkeys inoculated with virulent bubonic cultures die within the same period of time and from the same doses as do the corresponding animals inoculated with the pneumonic cultures. The same lesions are observed in animals after inoculation of the pneumonic strain as after the inoculation of the bubonic strain. Both strains when inoculated cutaneously, or subcutaneously, into guinea pigs and monkeys give rise to bubonic-plague infection. When the animals are infected by inhalation with either strain, similar lesions are also produced. In guinea pigs, after inhalation, infection results through the mucous membrane of the throat and upper portion of the respiratory tract, resulting in buboes of the cervical glands and septicæmia and in primary or secondary pneumonia; in monkeys, after infection by inhalation, primary pneumonic infection of the lung with secondary septicæmia results.

However, while during epidemics of bubonic plague reports

have been made that there is often a marked difference in virulence in the different cultures isolated, during this epidemic of pneumonic plague the organism seems to have retained a very high degree of virulence throughout. The cultures isolated from a number of cases near the close of the epidemic, upon inoculation into animals, proved to be fully as virulent and to kill animals as quickly and in the same doses as did those cultures isolated near the beginning. That the organism retained such a stable virulence throughout the epidemic is, perhaps, not surprising when one considers that infection occurred directly from man to man or, frequently one might say, from lung to lung and without the passage of the organism through rodents as ordinarily occurs in bubonic-plague infection. Morever, from the results of previous experiments relating to infection of animals with pneumonic plague by inhalation, we would expect that the organism would have retained its maximum virulence throughout this epidemic.

For these reasons and, also, from the fact that the acute course and mortality of the disease were not changed toward the close of the epidemic and especially, from the experimental proof furnished by the inoculation of animals with cultures isolated near the beginning and near the close of the epidemic, we must conclude that the sudden decline and cessation of the epidemic was not due to any marked change in the virulence of the strain. Such a decline and cessation must have depended upon other causes. The plague bacillus, whether isolated from pneumonic or from bubonic epidemics, usually exhibits marked stability in virulence. While it is not a very resistant organism in nature and easily becomes destroyed under certain conditions, it usually does not become markedly attenuated in passage through the animal body, and even on artificial culture-media, after many months, its virulence is usually fully retained. Instances of spontaneous loss of virulence in culture-media have been reported, but this is not usually the case with fresh, virulent cultures. This quality of stability of virulence of the plague bacillus, so different, for example, from that of the cholera vibrio, is of particular interest from an epidemiological standpoint.

AGGLUTINATION TESTS.

Theoretically the agglutination test has two applications in plague: (1) The diagnosis of the disease by the demonstration of antibodies in the patient's serum and (2) the identification of the organism cultivated from a suspected case by means of the serum of an animal immunized against the plague bacillus.

In pneumonic plague, the agglutination test has no clinical value, for the patients succumb to the disease before antibodies are produced or at least produced in any quantities that are capable of detection.

With regard to the second application of the method, as there seemed to be some difference of opinion as to the value of the agglutination test in identifying plague bacilli, we decided, after our return to Manila, to carry out a series of experiments in the hope of throwing further light upon this subject. The result of our experiments in this direction will be described briefly.

For obtaining an agglutinating serum, rabbits were used and large doses of the bacilli were injected intravenously. Repeated inoculation of *living avirulent* plague bacilli administered in this way called forth a very satisfactory serum, but *killed virulent* bacilli failed to do so in every instance. All of the rabbits lost weight rapidly during immunization.

After obtaining a satisfactory serum, the various pneumonic strains which we had brought back with us from Mukden, together with three bubonic strains and three strains from experimental animals, were all subjected to the agglutination test with the same plague serum. The organisms were grown at 32°C. In order to avoid spontaneous sedimentation, the bacteria were suspended in distilled water and the dilutions of the sera made with 0.1 per cent sodium chloride solution.

The greatest dilution of the immune serum, which caused complete or almost complete agglutination, is recorded for each strain tested in the following table:

TABLE II.—*Showing limit of agglutination in plague strains.*

Strain.	Limit of agglutination.	Strain.	Limit of agglutination.
Pneumonic plague:		Pneumonic plague—Continued.	
No. 1	160	No. 22	1,280
No. 5	160	No. 23	1,280
No. 7	640	No. 25	320
No. 8	320	No. 26	320
No. 9	160	No. 28	320
No. 10	160	No. 29	320
No. 12	640	No. 32	320
No. 13	160	Shanghai	320
No. 14	640	Hongkong	640
No. 15	320	Mariveles	320
No. 16	160	Avirulent plague	(?)
No. 17	320	Guinea pig:	
No. 18	640	No. 5635	80
No. 19	640	No. 5769	80
No. 21	640	No. 5745	80

Two points are strikingly obvious from this series of experiments: (1) There is great variability in the limits of agglutination of the different strains and (2) the strains freshly isolated from experimental animals agglutinate only at relatively low dilutions of the serum. It is also to be noted that both pneumonic strains and bubonic ones are agglutinated by the same serum.

It was next decided to select certain of these strains in order to make a careful study of the influence of various factors upon their agglutinability.

One of the difficulties in performing agglutination tests, particularly with strains which have been grown on artificial media for long periods of time, has been the tendency of the bacilli to form clumps spontaneously. We found that very small amounts of alkali would prevent this spontaneous flocculation and sedimentation of the suspensions, but on performing tests with such suspensions these extremely small amounts of alkali inhibited all agglutination.

It has already been mentioned that we performed a series of tests in the presence of only a small amount of electrolytes (0.1 per cent sodium chloride instead of 0.8 per cent). This served well to render the suspensions homogeneous and durable and agglutination was not inhibited, but there was frequently a disturbing flocculation with normal serum within very narrow limits, such as is often seen when one colloid is flocculated by another. As only two or three tubes of a long series were so affected, it was possible to distinguish between this phenomenon and the true specific agglutination. Nevertheless, the method was abandoned and in the tests to be described below both the bacterial suspensions and the dilutions of the serum were prepared with 0.5 per cent sodium chloride solution.

The following tables, selected from a number of similar ones with other strains of plague bacilli, demonstrate clearly the results of our experiments.

TABLE III.—*Showing agglutination of culture No. 26.*

	Grown at 37° C.			Grown at 32° C.			From guinea pig. Grown at 37° C.			From guinea pig. Grown at 32° C.			From guinea pig. Grown at 12° C.		
	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	3 hours.	20 hours.
Serum of immunized rabbit:															
1/5	++	+++	+++	+	++	+++	++	++	++	+	++	+++	trace	+	+++
1/10	++	+++	+++	+	++	+++	++	+++	+++	+	++	+++	trace	+	+++
1/20	+	+++	+++	+	++	+++	—	—	+	+	++	+++	trace	+	+++
1/50	—	—	trace	+	++	+++	—	—	—	+	++	+++	trace	+	+++
1/100	—	—	trace	+	++	+++	—	—	—	+	++	+++	trace	+	+++
1/200	—	—	—	+	++	+++	—	—	—	trace	++	+++	trace	+	+++
1/500	—	—	—	+	++	+++	—	—	—	—	trace	++	trace	+	+++
1/1000	—	—	—	trace	++	+++	—	—	—	—	trace	++	trace	+	+++
1/2000	—	—	—	trace	+	+++	—	—	—	—	trace	+	trace	+	+++
1/5000	—	—	—	—	+	+++	—	—	—	—	trace	+	—	+	+++
Salt solution	—	—	—	—	trace	+++	—	—	—	—	trace	+	—	+	+++
Normal rabbit serum:															
1/5	—	—	—	—	—	+++	—	—	—	—	—	trace	—	+	+++
1/10	—	—	—	—	—	+++	—	—	—	—	—	trace	—	+	+++
1/20	—	—	—	—	—	+++	—	—	—	—	—	trace	—	+	+++
1/50	—	—	—	—	—	+++	—	—	—	—	—	trace	—	+	+++
1/100	—	—	—	—	trace	+++	—	—	—	—	—	trace	—	+	+++
1/200	—	—	—	—	trace	+++	—	—	—	—	—	trace	—	+	+++
1/500	—	—	—	—	trace	+++	—	—	—	—	—	trace	—	+	+++
1/1000	—	—	—	—	trace	+++	—	—	—	—	trace	+	—	+	+++
Salt solution	—	—	—	—	trace	+++	—	—	—	—	trace	+	—	+	+++

TABLE IV.—*Showing agglutination of culture No. 12.*

	Grown at 37° C.			Grown at 32° C.			From guinea pig. Grown at 37° C.			From guinea pig. Grown at 32° C.			From guinea pig. Grown at 12° C.		
	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.
Serum of immunized rabbit:															
1/50	++	+++	+++	++	++	+++	++	+++	+++	+	++	+++	+	+	+++
1/25	++	+++	+++	++	++	+++	++	+++	+++	+	++	+++	+	+	+++
1/10	++	+++	+++	++	++	+++	—	+	++	+	++	+++	+	+	+++
1/500	trace	+	++	++	++	+++	—	—	—	trace	++	+++	+	+	+++
1/250	trace	+	++	+	++	+++	—	—	—	trace	+	+++	+	+	+++
1/100	trace	+	++	+	++	+++	—	—	—	—	trace	++	+	+	+++
1/5000	trace	+	+	+	++	+++	—	—	—	—	—	trace	+	+	+++
1/2500	—	—	—	+	+	+++	—	—	—	—	—	trace	+	+	+++
1/1250	—	—	—	+	+	+++	—	—	—	—	—	trace	trace	+	+++
Salt solution	—	—	—	—	trace	++	—	—	—	—	—	trace	trace	+	+++
Serum of normal rabbit:															
1/50	—	—	—	trace	trace	+	—	—	—	—	—	—	trace	+	+++
1/25	—	—	—	trace	trace	+	—	—	—	—	—	—	trace	+	+++
1/10	—	—	—	trace	trace	+	—	—	—	—	—	—	trace	+	+++
1/500	—	—	—	trace	trace	++	—	—	—	—	—	—	trace	+	+++
1/250	—	—	—	+	+	++	—	—	—	—	—	trace	trace	+	+++
1/100	—	—	—	+	+	++	—	—	—	—	—	trace	trace	+	+++
1/5000	—	—	—	trace	trace	++	—	—	—	—	—	trace	trace	+	+++
1/2500	—	—	—	—	trace	++	—	—	—	—	—	trace	trace	+	+++
Salt solution	—	—	—	—	trace	++	—	—	—	—	—	trace	trace	+	+++

TABLE V.—*Showing agglutination of culture "Hongkong."*

	Grown at 37° C.			Grown at 32° C.		
	2 hours.	4 hours.	20 hours.	2 hours.	4 hours.	20 hours.
Serum of immunized rabbit:						
1/10	++	++	+++	+	+	+++
1/20	++	++	+++	+	+	+++
1/40	+	++	+++	+	+	+++
1/80	+	++	+++	+	+	+++
1/160	+	++	+++	+	+	+++
1/320	+	++	+++	+	+	+++
1/640	+	++	+++	+	+	+++
1/1280	+	+	++	+	+	+++
1/2560	trace	+	++	+	+	+++
1/5120	trace	+	++	+	+	+++
Salt solution	—	+	+	+	+	+++
Serum of normal rabbit:						
1/10	—	—	—	—	trace	++
1/20	—	—	—	—	trace	++
1/40	—	—	—	—	+	++
1/80	—	—	+	—	+	+++
1/160	—	—	+	—	+	+++
1/320	—	+	+	+	+	+++
1/640	—	+	+	+	+	+++
1/1280	—	+	+	+	+	+++
Salt solution	—	+	+	+	+	+++

In Table III it is seen that the strain used agglutinates at greater dilutions when grown at 32°C. than when grown at 37°C. The control tubes with normal serum show in the case of the bacteria grown at 37°C. no agglutination whatsoever, even after twenty hours, while in the cases of the bacilli grown at 32°C., the bacteria have all settled out in twenty hours.

The same strain, after passage through an animal, does not agglutinate at as great dilutions as before. When grown at 32° C., it also shows less tendency toward spontaneous sedimentation than previous to the passage through the guinea pig. When grown at 12°C., flocculation begins almost as soon in the tubes with normal serum as it does in those containing immune serum, so that it is difficult to determine whether or not specific agglutination has taken place; however, after twenty hours, on shaking the tubes, the sediment in those with normal serum readily forms a homogeneous suspension, while in the first few tubes, at any rate of those containing immune serum, the sediment is seen to consist of coarser flocculi.

These same observations apply in a general way to the strain used in Table IV. This strain, however, when cultivated at

37°C. agglutinates at much greater dilutions of the same immune serum than does the previous strain when grown at this same temperature. Furthermore, the differences in agglutinability before and after passage through an animal are more marked.

The strain of Table V, when grown at 37°C., agglutinates at still greater dilutions. When cultivated at 32° C., flocculation takes place almost as quickly with normal serum as with immune serum, and it is difficult to decide whether or not specific agglutination has taken place.

Our strain of *avirulent plague* may be cited as the extreme of this series, showing varying grades of agglutinability. Even when cultivated at 37°C., spontaneous sedimentation takes place so rapidly that it is impossible to say that specific agglutination has occurred. Although this strain was used in producing the immune serum, we have in no single instance been able to show that it was agglutinated specifically by the serum.

The same immune serum was used for the tests recorded in Tables III, IV, and V.

We have previously noted that the plague bacillus forms more mucus when cultivated at 37°C. than when grown at 32°C. or even lower temperatures and that more homogeneous and more durable suspensions result in the former instance. We now see that the bacillus when grown at 37°C. is agglutinated with greater difficulty by a specific serum. It, therefore, seems not unlikely that the decrease in agglutinability is due to the increase in mucus production. In harmony with this view is also the fact that strains freshly isolated from experimental animals produce more mucus and are less readily agglutinated by a specific serum than are strains which have been cultivated for months upon artificial media, though the factor of possible difference in virulence also must be considered in this instance.[2]

Finally, we may add that during the course of these experiments we have been able to identify promptly by the agglutination test two strains which were isolated from bubonic cases of plague dying upon ships in the harbor of Manila.

While one of the difficulties in the performance of the agglutination test with the plague bacillus is the tendency toward spontaneous flocculation, we are inclined to believe that, under proper conditions, spontaneous flocculation usually does not occur in freshly-isolated strains; in most strains which have been grown upon artificial media for long periods of time it can be avoided by

[2] Strong, *Journ. Exp. Med.* (1905), 7, 229.

cultivating them at 37°C. The greater difficulty sometimes is, in our opinion, to obtain a satisfactory immune serum. We gave several rabbits repeated intravenous injections of large doses of killed virulent cultures without obtaining more than a trace of agglutination with their sera. We can strongly recommend the use of the living avirulent culture for the preparation of the immune serum.

In conclusion, we can only warn against the use of cultures grown at ice-box temperature as recommended by Shibayama[3] for the agglutination tests. Although such cultures are readily agglutinable, flocculation in the control tubes is apt to be very confusing. If one has a satisfactory immune serum, the culture grown at 32°C. or even at 37°C. will be agglutinated promptly and the control tubes will remain practically unchanged. Controls with normal serum should always be prepared in performing the test.

INFECTIVITY OF THE EXCRETA.

In no other disease is the infecting organism found in such abundance in the sputum as it is in pneumonic plague. When the disease is well developed, *Bacillus pestis* is present in almost pure culture. In pneumonic plague as in bubonic plague, when the disease becomes septicæmic, the organisms are sometimes found in the urine and even sometimes in the fæces. When once the sputum of pneumonic-plague cases becomes thoroughly dried it is no longer infectious, but when the sputum becomes frozen and pulverized, particles of it may be blown about and remain infective for long periods of time or until the sputum is again thawed.

BACTERIOLOGICAL DIAGNOSIS OF PNEUMONIC PLAGUE.

EXAMINATION OF THE SPUTUM.

A bacteriological diagnosis from the sputum can not be made at the onset of the disease, and not until after the fever has developed does the sputum appear. Shortly after the appearance of the sputum, the plague-organism, even if not visible from the microscopical examination, may be isolated by culture. When the sputum becomes bloody, the organism is usually present in large numbers and in almost pure culture. Sometimes the organism might be mistaken morphologically for *Diplococcus pneumoniæ*, and bipolar-staining organisms, other than plague bacilli, may

[3] *Centralbl. f. Bakt. etc.*, Orig. (1905), 1 Abt. 38, 482.

sometimes be encountered in the sputum. While in the microscopical examination of the sputum Gram's stain is a very valuable aid in arriving at a diagnosis of the organism, nevertheless, Gram-negative bacilli have been encountered in the sputum, which proved later not to be plague bacilli. However, usually if the sputum is blood-stained, from the microscopical examination, with the aid of Gram's stain, there is no difficulty in arriving at a diagnosis, since the plague organism is usually present in such very large numbers. In the later stages of the disease, involution forms are commonly encountered in the sputum. The organisms are constantly found in great abundance up to the time of death.

BACTERIOLOGICAL EXAMINATION OF THE BLOOD.

In the early stages of the disease, cultures from the blood are frequently negative. Sometimes, however, the organism could be cultivated from the blood from twenty-four to forty-eight hours before death, and it could always be obtained from the blood a few hours before death. In many instances the bacteria are present in very large numbers in the blood, so that a diagnosis can often be made from a simple, microscopical examination. In no other disease is so marked a bacteræmia present. In the early stages of the disease, cultures from the blood should be made in bouillon, as much as 1 cubic centimeter of blood being employed. The agglutination test is of no value in making a diagnosis, as the course of the disease is too acute and the patient has succumbed before the agglutinins appear in demonstrable quantities. The reaction of the deflection of the complement is also not to be recommended for the same reason; the examination of the sputum and blood for the presence of the bacillus gives much greater and more valuable information. In cases where no necropsy is permitted and a post-mortem bacteriological diagnosis is advisable, microscopical examination of material, obtained by lung puncture with a syringe, may often be conclusive of pneumonic plague, *Bacillus pestis* being present in the microscopical preparation, in enormous numbers, in pneumonic-plague cases.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

VII. PATHOLOGY.

By RICHARD P. STRONG, B. C. CROWELL, AND OSCAR TEAGUE.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Although bubonic plague is a disease that has occurred in large and protracted epidemics and has been widely studied, epidemics of pneumonic plague, even of moderate size, have not been frequent and very few contributions to the literature upon the pathological anatomy of primary pneumonic plague have hitherto been made. Moreover, none of these articles has been based upon the study of extensive material during a large epidemic, and some of the cases described in the literature as those of primary pneumonic plague are really instances of secondary infection of the lung. Therefore, the subject of epidemic pneumonic plague is one of particular importance in connection with the Manchurian epidemic.

Hitherto, our ideas regarding the pathology of pneumonic plague have been based largely upon several cases described by Childe in 1897–1898, and upon three cases reported by Albrecht and Ghon in 1898. The German Plague Commission (Gaffky, Pfeiffer, Dieudonné, Sticker) in the report of their investigations in India, during the same year, described 7 cases of pneumonia in plague, but when one reads the description of these, it is found that but two were cases of primary pneumonic plague, and in both of them the infection was complicated by the presence of other bacteria, in addition to the plague bacillus.

Childe,[1] in 1897 and 1898, describes the post-mortem lesions in two cases of pneumonic plague. In the first case the necropsy was performed seven hours after death and the lesions encountered are described as follows:

"The lungs showed much general engorgement and œdema, with serosanguineous frothy fluid in the bronchi, but no pus; the usual appearances of acute bronchitis were absent. There was one small pneumonic patch, the size of a walnut, in the early second stage, situated below the apex

[1] *Brit. Med. Journ.* (1897), 1, 1215.

on the front of the right lung, and two similar but smaller patches at the same part of the left lung; these patches stood out a little from the surface, and were airless, friable, and sank in water, each was surrounded by a dark ring of engorgement, which merged into the healthy lung, and there was recent pleurisy over the pneumonic areas. All the other organs were examined, and showed considerable engorgement, but no special lesion was observed. The cervical, the axillary, and the lumbar lymphatic glands were slightly enlarged; the left iliac slightly enlarged, red, and soft; all the other glands, including the bronchial, looked absolutely normal."

Childe states that he had performed 12 post-mortem examinations on pneumonic-plague cases all presenting appearances similar to those described in the one above.

In his later publication in 1898,[3] he describes the pneumonic form of plague as follows:

"In this form of plague, the only marked evidences of disease are found in the lungs; the lymphatic glands and other organs are scarcely affected at all.

"*The Condition of the Lungs.*—There was general engorgement with considerable œdema, a reddened condition of the mucous membrane of the bronchi, but no marked evidences of bronchitis, and frothy watery fluid, sometimes blood-stained, could be squeezed from the bronchi. (Pus in the bronchial tubes was only found on one occasion.) A number of pneumonic patches were found scattered through the lungs, varying in size from a pea to an egg. They were light pink or red-grey in colour, solid, airless, and sank in water; they were rounded in shape, and usually separated by a distinct ring of engorgement from the crepitant lung around. Some, instead of being pink, were of a deep blood colour throughout, and less solid, and some of these had a small, greyish, more solid centre. Those of the patches which were situated on the surface of the lung were prominent, and projected distinctly from the surface; whilst the pleura over them was roughened, and showed signs of early inflammation. These patches had, in fact, the appearance of the first and second stages of lobular pneumonia, but no patches were found which had passed on to the third stage of softening and breaking down. In a few cases larger masses of pneumonic lung than these were found, and once about half the lower lung was found in this condition. Petechial hæmorrhages were usually found on the surface of the lung; the bronchial glands were either enlarged, swollen, œdematous, soft, and distinctly engorged, or else they were small and of the usual appearance, perhaps a little engorged. The remaining lymphatic glands throughout the body showed none of the appearances of either the bubonic or septicæmic form of plague; most of them looked absolutely normal, and the only noticeable change was that the axillary, and sometimes the cervical, chains were a little engorged.

The description of the remaining internal organs already given applies equally to this form of plague, except that the large hæmorrhages were absent, but petechiæ on the surface of the heart, in the pelvis of the kidney, bladder, stomach, and intestines were commonly present. Petechiæ in the skin were not observed in this form of plague. * * *

"A section of lung tissue, apart from a pneumonic area, shows great engorgement of all large blood vessels, and of the alveolar capillaries as

[3] *Ibid.* (1898), 2, 859, 860.

well, and patches of hæmorrhage into the alveoli around these engorged vessels are seen scattered about. In a pneumonic area three zones can be made out. At the circumference there is intense engorgement of all vessels including alveolar capillaries, the alveoli are full of blood, and the hæmorrhage is so intense that many of the alveolar septa are broken down, entirely absent, or represented by mere shreds. Within the circumference is seen a zone in which the alveoli are intact and are completely filled with well-stained cells, so that there is no interval between the alveolar walls and their contents; and at the centre is one universal mass of similar cells, and the cellular infiltration is so extreme that the walls of the alveoli are scarcely visible. Such is the general arrangement of the pneumonic patch, although there may be alveolar hæmorrhage in parts of either the middle or central zone. Under a higher power the alveoli of the circumference are seen to be completely filled with blood corpuscles, and there is scarcely an appearance of fibrin, or none at all; in the middle zone the alveolar contents consist for the most part of catarrhal epithelium with some white and a few red blood corpuscles, and a little fibrin or none at all, whilst the dense central mass of the cells consist of catarrhal epithelium and leucocytes with some granular *débris*. Thus the pneumonic area has the appearance of very extreme lobular or catarrhal pneumonia. The walls of the bronchial tubes, as well as of the large veins, show great engorgement and there are hæmorrhages into the vein walls. Blood and catarrhal cells may be seen in the finer bronchi, but the bronchial mucous membrane is scarcely altered, there being at most a little cellular proliferation. There are the appearances of acute pleurisy over those pneumonic areas which project upon the surface of the lung, with hæmorrhages beneath the pleura. The bronchial glands show engorgement of blood vessels, some hæmorrhage into the gland tissue and distended lymphatic vessels; but in some cases these conditions are only slightly marked and the glands looked nearly normal."

Albrecht and Ghon[3] in their report upon bubonic plague describe three cases of primary plague pneumonia. From the study of these cases, they concluded that the primary plague pneumonia represents a typical lobular pneumonia or bronchopneumonia which involves either a single lobe, or several lobes (in some cases bilateral), or an entire lung.

They further state: On the cut section one can, as a rule, still make out the confluence of the separate infiltrated lobules, since their boundaries can still be partially distinguished. The posterior portions of the lung tissue are most often attacked by the inflammation. It has already been remarked that the primary as well as the secondary plague pneumonias both have a very characteristic and, in this sense, specific appearance, since the finer anatomic picture resembles that of no other inflammatory disease of the lungs with which we are acquainted. Even in the pleura the peculiar conformation and color of such foci is striking. The pleura is either only slightly cloudy, injected to a bright color, and dotted with numerous small hæmorrhages, or it is covered with or penetrated by a yellowish, fibrinous, exudative membrane. This fresh pleurisy is, as in every pneumonia, a regular part of the inflammatory process.

[3] Ueber die Beulenpest in Bombay im Jahre 1897. K. Akad. d. Wiss. (1898), II. B., 429.

One sees beneath the pleura fine yellow and red dots and spots caused by numerous yellow nodules or bands upon a bright red background. The picture resembles exactly the one encountered in many lymph glands which contain numerous bacilli. Microscopical sections show that this picture is due above all to the fact that the distended alveoli are filled with enormous masses of bacilli or with blood and with these almost alone; the cut section shows a similar, generally mixed yellow-red color, appears as though most finely shagreened but never really granular, and yields an abundant, somewhat viscid juice.

The changes in the septa of the alveoli are very characteristic and indeed as well in the primary as in the secondary pneumonias. The septa are very strikingly broadened and changed into a glistening frame-work which is sometimes coarser, sometimes more thread-like, and stains well with eosin. Between the bands of this frame-work are enclosed, in scant numbers, cells or cell nuclei or red blood-cells: the thick cords are lined by small and most minute granules standing close together and by cell nuclei, irregular, pear-like, or spermatozoon-like in shape.

The complete agreement with the changes in the vessels of primary buboes and with the multiple foci in the spleen is obvious at the first glance. The large numbers of plague bacilli in the alveoli lead also in this case to that peculiar coagulation of the tissue-fluid and the cellular elements of the septa of the alveoli and the vessel-walls; at the same time coagulation takes place in the blood within the vessels. The finer or coarser bands, which thus arise, do not give the fibrin-staining reaction of Weigert.

In addition to these changes in the septa of the alveoli, which are to be regarded as due to necrosis, there appears at places a complete disappearance of the septa, so that only spur-like remnants of the same are left. The bronchioles are also markedly dilated and filled with enormous masses of bacilli, which occur also in just as large numbers in the large bronchi and are of course expectorated. However, fibrinous exudation is everywhere almost completely lacking, only a scant fibrin network being found here and there. The amazingly large number of plague bacilli is also evident from cover-slip preparations and from cultures from the pneumonic lungs. In primary plague pneumonias, we found plague bacilli twice in pure culture and once mixed with a small number of diplococci (*Diplococcus pneumoniæ*), in the metastatic foci only once in pure culture and three times with diplococci, which were also in these cases not numerous. We, of course, found a mixture of different bacteria in the pneumonic foci due to aspiration.

With regard to the frequency of the occurrence of pneumonic foci due to the plague bacillus, among 44 acute plague cases we found such foci nine times; viz., 3 primary plague pneumonias, 4 metastatic or secondary plague pneumonias, and 2 aspiration pneumonias in which we could demonstrate numerous plague bacilli. The metastatic plague foci in the lungs hence occur rather seldom if one considers that undoubtedly the circulation is flooded with plague bacilli, either only for a short time or frequently several days before death.

The Anglo-Indian Plague Commission[1] report "that the lesions in pri-

[1] Report of the Indian Plague Commission (1901), 5, 435.

mary pneumonic plague, when contrasted with those occurring in *Pestis major*, are less intense in the other organs, with the exception of the lungs.

"The lymphatic glands are only slightly affected, and external buboes having the specific characteristics seen in *Pestis major* are seldom, if ever, encountered. Congestion and enlargement of organs and even hæmorrhage in mucous and serous membranes may be present, but they do not assume the proportions attained in *Pestis major*. On the other hand, the lungs are conspicuously affected. The whole substance is engorged, the large as well as the small blood vessels being distended; and hæmorrhagic zones are seen scattered throughout the lungs, filling the alveoli and often breaking down their walls. Within the hæmorrhagic zones are areas in which the alveoli are completely filled with leucocytes, epithelial cells, and granular débris, constituting, with the surrounding zones of hæmorrhage, blood-congested areas of catarrhal pneumonia. In these areas, as well as in the fluid matter contained in the trachea and bronchi, plague bacilli are abundantly present. Greyish necrotic patches have also been found containing large numbers of plague bacilli. The bronchi are engorged with blood, and catarrhal cells are found in their terminations. Over affected areas at the surface of the lungs, the pleura may be acutely inflamed. In most cases, the bronchial glands were congested, and there was a little hæmorrhage into the gland substance; but in some cases, their appearance was normal.

"While, however, a catarrhal inflammation of lobular distribution has most frequently been regarded as the characteristic type of primary plague pneumonia, several observers have denied its existence, and have asserted that croupous (lobar) pneumonia is the form that most frequently occurs. Major Evans, I. M. S., and Captain Elphick considered that all cases of typical plague pneumonia come under the latter category, and Major Jones expresses the opinion that "lobar pneumonia is common." Major Evans stated that the pneumonia is distributed in small detached patches, constituting lobular areas, only when the inflammation has not advanced far; but that it is lobar to the extent of involving a whole lobe or the greater part of a lobe when the lung inflammation has advanced further. Captain Elphick, I. M. S., described several autopsies in which individual lobes or even an entire lung was consolidated, and he stated that "every case of pneumonic plague examined showed lobar condensation." It may further be stated that in many cases only slight changes were found in the bronchi. It is therefore possible that the pneumonia is lobular in patients who have died at an early stage of the disease, and lobar in those who have survived to a later period; or, otherwise, that lobar pneumonia occurs when the toxin is most virulent and most widely distributed throughout the lung, and lobular pneumonia when it is less virulent and less widely diffused.

"* * * [The] microscopic examination has mainly shown general dilatation and engorgement of the veins and smaller blood vessels and numerous capillary and larger hæmorrhages in almost every structure and organ of the body."

Dürck[5] and Herzog[6] have reported at some length upon the general

[5] Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie (1904–5), Supp. 6–7.

[6] *Pub. Bur. Govt. Labs.* (1904), No. 23, 9.

pathological anatomy of bubonic plague, but neither of these observers had any special opportunity for the study of the primary pneumonic form.

During the Japanese epidemic of 1899, reported by Kitasato, Takaki, Shiga, and Moriya,[7] 13 cases of primary pneumonic plague occurred, but no necropsies were made. Sata[8] has recently reported upon the pathological anatomy of a single case of pneumonic plague in which, however, the lesions were complicated by the presence of other bacteria, besides the plague bacillus.

On account of this absence in the literature of observations upon the pathological anatomy of *epidemic* pneumonic plague, the results of the study of this subject made by us during the Manchurian epidemic will be reported in detail.

Our observations upon the human pathology of this disease are based upon the study of 25 complete necropsies performed at the plague hospital at Mukden. All of the material was fresh, many of the necropsies having been performed immediately or within a few hours after death.

The histological examination of the tissues has been performed in Manila since our return. Zenker's fixation with alcohol preservation of sections was employed in all cases, while primary alcohol fixation of sections from some cases was also used in order to facilitate bacterial investigation. It was necessary in a few cases to resort to the study of the material which had been preserved in Kaiserling's fluid. All tissues were sectioned in paraffin and stained with Böhmer's hæmotoxylin and eosin; in addition Weigert's stain for fibrin, Unna's methylene blue and eosin, the Gram-Weigert stain, and Mallory's iron hæmotoxylin were used as differential stains in nearly all of the cases.

It has been deemed advisable to consider the gross and histological lesions under the description of each organ.

External appearance.—The bodies with one exception were those of robust, well-nourished individuals and showed no emaciation. Two of the subjects showed evidences of old syphilitic infection and one had early carcinoma. None of them was tuberculous. The superficial lymphatic glands were not enlarged, and carbuncles, vesicles of the skin, or buboes were not observed. Small punctiform hæmorrhages in the skin about the bends of the elbows and over the chest occurred in two cases and were apparently the result of needling.[9] *Livor mortis* was not as a

[7] Bericht über die Pestepidemie in Kobe und Osaka. Tokio (1900).

[8] Quoted by N. Masuyama, *Ztschr. f. klin. Med.* (1910), 70, 498.

[9] A method of treatment of the disease employed by certain Chinese physicians of the old school.

rule very extensive or marked owing to the freshness of the cases; in three it was extensive over the shoulders and the dependent parts and was of a dark, brownish-red color. *Rigor mortis* in some of the cases had not developed. In others it was very strong. In degree it was, perhaps, when compared with the rigor mortis occurring in other acute infectious diseases, only surpassed by that seen in Asiatic cholera. The muscles were usually of a bright-red color; hæmorrhages were not observed in the abdominal ones, but small extravasations of blood were on one occasion noted in the thoracic muscles in stripping them from the thoracic wall and ribs.

Pericardial cavity, heart and blood vessels.—In the anterior mediastinum in the tissues surrounding the thymus gland usually much œdema and frequently extensive hæmorrhages occurred. On the visceral surface of the serous layer of the pericardium, petechiæ often occurred and larger punctiform hæmorrhages were sometimes encountered. On the epicardium varying numbers of petechiæ were observed in most of the cases. The right chambers of the heart were usually distended with blood and in a number of cases showed acute dilatation and thinning of the wall, particularly of the right auricle. The muscle was in some instances soft but usually of a fairly firm consistency. Cloudy swelling was almost invariably noted; early fatty degeneration was observed in a few instances. The bronchial veins sometimes showed hæmorrhages in the intima, and numerous extravasations of blood occurred about the vessels posterior to the peritoneum and in the region of the kidneys, omentum, and mesentery. In the omentum, hæmorrhages were particularly observed in the fat around the larger veins.

Histological examination of the heart.—The changes in the heart consist chiefly in a cloudy swelling of the muscle fibers with some œdema between the fibers in some cases. The fibers are, however, usually closely packed. In some cases slight hæmorrhage was present beneath the epicardium. In some there was infiltration of the epicardial fat into the muscle, and in one or two cases slight infiltration of this fat between the muscle bundles.

Fatty degeneration of the fibers to any marked degree was not noted, but lesser degrees could not be determined on account of the fact that the method of preservation of the tissues did not permit of the satisfactory use of selective stains for fat.

Fragmentation of the fibers was a constant feature in all of the cases examined. No exudation from the vessels was encountered, although the vessels were constantly engorged.

Pleuræ.—The parietal pleura covering the thoracic wall, diaphragm, and pericardium in many instances showed numerous ecchymoses in the region of the infected lung, and very often delicate, fibrinous adhesions were observed between the parietal and visceral pleuræ. In some instances many of the hæmorrhages were punctiform in character, but in others they were confluent and formed diffuse, larger, dark-red patches.

Lungs.—Numerous ecchymoses beneath the pleura were almost always encountered, though they varied greatly in extent and in number. The appearance of the lungs varied according to the stage of the disease at the time of death. Generally the lungs were dark red, voluminous, very rich in blood, and very œdematous. From a careful study of the lesions of the lungs we can conclude that plague pneumonia is an anatomically defined type of disease different from other varieties of pneumonia.

Fresh, fibrinous pleurisy was observed in every case, extending over the more marked areas of pneumonia. (See Plates IX and X.) In some instances the delicate membrane was reddish and slightly roughened; in other cases, grayish or grayish white or yellowish, and could be easily peeled from the surface of the lung. Rarely a gelatinous, œdematous exudate was present. In two instances, the pleural cavity contained between 100 and 200 cubic centimeters of a serous hæmorrhagic exudate in which large numbers of plague bacilli were present.

Some portion of the lung showed either inflammatory engorgement or pneumonic infiltration. The seat of the pneumonia varied greatly. The upper lobes appeared to be quite as frequently involved as the lower.

On section of the lung, the tissues adjacent to the areas showing pneumonic involvement usually revealed very marked congestion and œdema. Such areas were firmer than the normal lung and tore more easily. On pressure, a reddish, serous fluid exuded from the cut surface in great abundance. Sometimes in these areas the œdema was so great as to give to the lung tissue a jelly-like consistency.

While in croupous pneumonia the first stage of inflammatory engorgement as an independent condition is almost never, or certainly very rarely, encountered, as the patient does not succumb within twenty-four to thirty-six hours from the origin of the disease, in our cases of plague infection of the lung the early stages of inflammatory engorgement were frequently met with and often death occurred before the lesion had progressed further, so that indeed in some instances no true pneumonia was yet

visible. In the stage of inflammatory engorgement, the plague-infected lung was voluminous, firmer, and less crepitant than the normal lung, and either dark red or reddish blue in color. Upon section, the tissues were found to be very œdematous, and a thin, reddish serum escaped in great abundance.

The pneumonic areas, when present, were either lobular or lobar in type. (See Plates VIII, IX, and X.)

In the lobular type, one or several nodules varying from about three to five centimeters in diameter might be found in the lobe. They were rather sharply circumscribed from the surrounding lung tissue by a more or less marked ring of engorged pulmonary tissue and were either circular in outline or wedge-shaped. In one instance, on section of the lung, six areas in the stage of early gray hepatization were observed in one lobe situated near the base. Three of these measured 2, 1.5, and 1 centimeters in diameter and were all arranged along the same bronchus. About one-half centimeter from the tip of the base of the lobe on the same bronchus were three more hepatized patches measuring 5 or 6 millimeters in diameter. The mucous membrane of the bronchi was dark red in color. The other lobe and the right lung in this case showed only congestion and œdema. Sometimes these pneumonic areas are situated on the surface of the lung, when they project distinctly from the surrounding lung tissue, and the pleura over them is roughened and shows other signs of early inflammation. Such areas of broncho-pneumonia as just described no longer contained air. On cut section the surface was rather dry, grayish red in color, and finely granular in appearance. No muco-purulent secretion was visible in the smaller bronchi, and, on pressure, mucus plugs were not expressed from the bronchi as is frequently the case in bronchial pneumonia due to infection with other microörganisms. The granular appearance of these areas is not identical with that observed in croupous pneumonia. The granules are irregular and coarser, and, on scraping the surface of these areas with the knife, no fibrin plugs are observed to escape from the air cells, but the juice so expressed is grayish white, slightly sticky, and evidently highly albuminous. The alveolar septa sometimes appear broader than normal. The mucous membrane of the bronchi leading to such areas was bright red in color. Occasionally several pneumonic areas might be arranged along one bronchus somewhat as the flowers of the hydrangea are placed on the stalk of the plant.

While a careful study of the human lungs, as well as of those

of numerous monkeys and guinea pigs, in which pneumonic plague had been produced by inhalation of plague bacilli, has shown that the pneumonia is primarily bronchial in origin and of the lobular type,[10] nevertheless, very early lobar involvement was very much more frequently encountered in the human cases at necropsy.

In the lobar type, the whole lobe or a portion of it showed either only early inflammatory engorgement or a portion of the lobe early red, with beginning gray hepatization. Plate X illustrates a section of the lung in the stage of gray hepatization. We have not seen an entire lobe in the stage of gray hepatization such as is frequently observed in ordinary croupous pneumonia due to the *Diplococcus pneumoniæ*, as the patients with primary pneumonic plague evidently die before this stage is ever reached. Large areas of red hepatization are also rare. However, a smaller area of gray hepatization, adjoining one of red hepatization and this in turn shading into an area showing only engorgement, was sometimes observed. Very frequently death evidently occurred before any apparent hepatization had taken place and only a portion of a lobe showed engorgement. Even in these instances, however, enormous numbers of plague bacilli were present in the lung tissue. In but comparatively few of the cases had the stage of gray hepatization been reached, and evidences of resolution were not encountered.

Rarely one lung was practically normal in appearance. However, in these cases in which one lung only showed pneumonic infiltration, the other usually showed congestion and œdema. In other instances single lobes, or all the lobes of one lung, might show early inflammatory engorgement. In some of the cases both types of pneumonia were encountered. In one lung the lobular areas might be observed while in the other the appearance of a lobar type was present. Or in the same lung a smaller area of gray or red hepatization might be encountered while the remainder or some part of the lobe showed pneumonic infiltration in the stage of engorgement. The differentiation of the so-called lobar type of pneumonic plague from other varieties of pneumonia may not be as easily accomplished as in the case of the lobular type. The cut surface of the pneumonic lung, however, in the stage of early gray or red hepatization, usually seems less granular; the condition of the bronchi and

[10] See also IV, p. 173 of this report.

the character of the exudate also renders assistance in arriving at a diagnosis. Also the absence or scarcity of fibrin in the alveolar exudate in pneumonic plague is in striking contrast to the condition observed in croupous pneumonia. The alveoli are frequently filled with plague bacilli. The gross lesions of the human lung are illustrated in Plates VIII, IX, and X.

Histological examination.—No cases in the series were found in which the lungs exhibited no alteration and no part of any lung examined was free from at least some pathological changes. In the earlier cases (1) the presence of bacteria, (2) the changes in the blood content of the vessels, and (3) the changes in the bronchi and bronchioles constitute the prominent features.

(1) The bacteria occur in enormous numbers and frequently appear as dense blue clouds in thin sections stained by hæmotoxylin and eosin and examined even with a low magnification. In general, this method of examination gives the most satisfactory evidence of the distribution of the bacteria, and the higher magnification with the oil-immersion objective is only necessary when the bacteria must be searched for.

In the earliest cases the bacteria are especially numerous about the bronchioles, in the peribronchial lymph spaces, and adjoining alveoli. Here they frequently form masses completely encircling the bronchioles, and are also present in large numbers in the interlobular septa and beneath the pleura. In the lungs which are the seat of anthracotic deposits, wherever anthracotic pigmentation is found, there are enormous masses of the bacteria, and the distribution of the bacilli about the bronchioles, in the interlobular septa, and beneath the pleura is recognized as the usual distribution of anthracotic pigments. In these earlier cases there are but few bacilli in the blood contained within the vessels and also few in the neighborhood of the vessels. The alveoli in this stage also contain bacilli when there is no recognizable change in their lining epithelium.

(2) The blood vessels in the interalveolar and interlobular septa are widely distended with blood and occasionally small hæmorrhages have taken place from these vessels into the alveoli. Very few, if any, bacteria can be found in the blood vessels in the earlier cases.

(3) The smaller bronchi and bronchioles are in the condition of catarrhal inflammation. The lining cells are swollen and frequently desquamated, and there are some few red blood-cells and leucocytes among the lining cells and in the lumina of the bronchioles. Mucus in the form of granular flakes is also con-

tained in the bronchi, as well as very large numbers of bacilli. The distribution of the bacilli about the bronchioles has already been described.

The condition of the lung alveoli in the early stage of the disease varies in different areas. The septa have already been referred to as engorged and the alveoli about the bronchi as filled with bacilli. The alveolar epithelium is swollen and frequently desquamated in the form of large cells often containing abundant pigment. The contents of the alveoli consist of a few of these desquamated cells, serum, bacilli, and an occasional leucocyte or red blood-cell.

The pleura may be the seat of a slight fibrinous exudate, and small hæmorrhages from the vessels may have occurred.

As the process passes on to the later stages of the disease, the added features are those of exudation and more extensive hæmorrhages.

The exudation consists in the passage into the alveoli of red blood-cells and leucocytes, the stage where the red cells predominate probably preceding that of the mixed red and white exudate, so that a red and a white stage can be differentiated. But in no case does the latter condition proceed so far as in the ordinary pneumonia due to *Diplococcus pneumoniæ* and the leucocytes are never so abundant. That is to say, there is not a pure white stage, as a good proportion of red cells is always present and the red appearance of the lung is also more prominent on account of the frequency of hæmorrhages which may be small or involve a large portion of the lung. The leucocytes are chiefly of the polymorphonuclear neutrophile type, although some mononuclear cells are present. Few, if any, eosinophiles are present in the exudate. Fragmentation of the nuclei of the leucocytes is not infrequent. A peculiarity of the leucocytes when seen under high magnification is that they are very frequently surrounded by a clear zone. The possibility of specific staining for fat was precluded by the method of preservation of the tissues. Phagocytosis was seldom observed.

The presence of fibrin in the exudate is an unusual occurrence, and in those cases where careful staining showed it to be present, the amount was in no way comparable to the amount found in ordinary pneumococcus pneumonia and when present was only at the immediate periphery of the alveoli in the neighborhood of the vessels.

In the later stages of the disease, the bacteria are very numerous and here, in contrast to what was described in the

earlier cases, they are in greatest number about the medium-sized vessels and can also be seen in the blood contained within the vessels. They are also present in large numbers immediately beneath the pleura and in the fibrinous exudate which always covers the pleura over a consolidated area. Where sections were taken through the interlobar septa, the presence of a leucocytic exudate can be seen in the groove between the pleural surfaces. (See Plates XIII to XVII illustrating histological changes.)

The bronchi.—(See Plate VIII.) The mucous membrane of the bronchi was in every case of a bright-red color which varied in the different instances only in intensity. Often in the bronchi near the bifurcation, the deeper red portions appeared as closely placed, parallel, longitudinal stripes in the bronchial wall. The bronchi contained a red, frothy, bloody serous fluid or more rarely a reddish mucus exudate. The yellow or whitish muco-purulent exudate frequently seen in cases of catarrhal bronchitis or in other forms of pneumonia was never observed, nor were fibrinous plugs encountered. In one case in which the lesions in the lung as well as the changes in the other organs were not very far advanced, the diagnosis of primary lung infection with plague bacilli was suggested from this condition in the bronchi and the character of the exudate. This diagnosis was confirmed by bacteriological examination.

Pharynx, larynx, and trachea.—(See Plate XI.) The anterior surface of the tongue was usually coated with a brown, buff, or pinkish-gray layer. The papillæ at the base of the tongue and the lymphoid follicles here and on the posterior wall of the pharynx were swollen. The tonsils were in every instance of about normal size or slightly swollen. On cut section the surface was usually reddish or reddish gray and in a few instances bluish in color. In only one case were there small areas of necrosis and hæmorrhages in the tonsil. The mucous membrane of the mouth and throat over the base of the tongue, uvula, tonsils, and adjacent structures was in all cases somewhat swollen and generally appeared of a more or less congested, dark-red color or in a few instances of a reddish-purple hue. From the pharynx to the larynx the mucous membrane as a rule gradually assumed a brighter red color. Over the epiglottis, vocal cords, cartilages, and whole larynx it was generally markedly hyperæmic and red in color, but in a few instances of a whitish-pink or pink hue. When the color was not bright red the injected vessels could be seen more plainly upon the pink background just described. The

mucous membrane just above the vocal cords in a few cases was not so markedly hyperæmic, but below them, in every instance, it appeared of a bright-red color. Throughout the entire length of the trachea the hyperæmia was always more marked below the vocal cords, whatever the condition above them was. This hyperæmic condition continued in every instance throughout the trachea and bronchi, though it was sometimes less marked in the smaller tubes which led to normal lobes of the lung. In no case was there noticeable œdema of the glottis. In a single instance, in which the epiglottis and surrounding structures showed no injection, the hyperæmia and injection of the vessels did not begin until about 3 centimeters below the cords. In a few cases there were small hæmorrhages measuring several millimeters in diameter in the mucous membrane of the trachea. Over the surface of the trachea a small quantity of blood-stained serous exudate, sometimes frothy in character, was present. There was always much œdema of the tissues surrounding the lower portion of the trachea, and the lymphatic glands in this region were swollen to a greater or less degree. In one instance two of them measured as much as 3 centimeters long by 1½ wide. (See Plate XI.) On cut section they were usually red or bluish in color and showed many hæmorrhages. The glands at the bifurcation of the trachea were always greatly swollen, generally anthracotic, and in all instances were of an almost black-red color from resulting hæmorrhages in the glandular substance.

Histological examination of the tonsils.—The morphological changes in the tonsils are not prominent. The majority of the tonsils examined did show very marked congestion and in some œdema was present. Small hæmorrhages were noted in a few cases. The crypts frequently contained mucus in which very moderate numbers of pest bacilli were present. The epithelium and follicles for the most part showed no change. Some of the tonsils were the seat of old inflammatory changes which were unrelated to the plague infection. One case showed very active proliferation of the lymphoid follicles. This case showed very few plague bacilli in the tonsil itself but the greatest numbers observed were present in the blood contained within the vessels of the tonsil. The majority showed very few bacilli at any place in the tonsil.

One showed very large numbers of pest bacilli in the crypts and scattered throughout the parenchyma, without any definite distribution, except that the follicles were practically free.

Another case showed a remarkable apparent leucocytosis judging from the number of leucocytes in the blood within the vessels.

The remarkable feature about the tonsils as a whole was their comparative freedom from anatomical changes. Congestion, sometimes œdema, sometimes hæmorrhages, occasionally slight lymphoid activity constituted the main features. (See Plate XVIII.) The bacilli were present in small numbers with one exception, and never bore any comparison to the number in the lungs.

The *œsophagus* was in every instance normal, no hyperæmia of the mucous membrane being observed.

Stomach and intestines.—The mucosa of the stomach was frequently somewhat swollen and showed numerous, small ecchymoses. In a few instances small erosions were present. In a few cases the peritoneal surface of the small intestine was reddened and in a few others hæmorrhages were observed on the peritoneal surface of both the large and small intestines. These hæmorrhages were of two types—the first dark, almost black in color, measuring from ½ to 1 centimeter in diameter and suggesting in their appearance *œsophagostomum* infection; and the second appearing as fine, bright-red, linear hæmorrhages. The mucous membrane in these cases was reddened and showed a catarrhal condition, with a pinkish mucous layer covering the surface, beneath which were innumerable, bright-red, pin-point-sized areas.

Lymphatic glands.—The bronchial glands near the bifurcation of the trachea always showed more advanced changes than any of the other lymphatics; they were always swollen, rich in blood, and frequently almost black in color from resulting hæmorrhages. The lymphatics along the lower portion of the trachea were also usually swollen and sometimes contained hæmorrhages. In a few cases the mesenteric lymphatics showed simple inflammatory swelling, but in the majority of the cases they were normal. The largest ones measured about 2½ centimeters in diameter. On section the surface was pink or of a grayish-red or dark-red color, but showed no hæmorrhages or necrotic areas, although in one case in the veins about them the blood had frequently escaped from the vessel walls. In one instance the glands showed small hæmorrhages. In the other lymphatics of the body, no special changes were observed.

Spleen.—The spleen was distinctly enlarged in 56 per cent of the cases. In bubonic plague the percentage with distinct anatomical enlargement of the spleen is considerably higher, but the spleen is by no means always enlarged in bubonic plague, as has frequently been stated. In the present cases it was usually

firmer than the typical, infectious splenic tumor, a condition depending upon the increase of red pulp and blood in the organ. On cut section the red pulp was greatly increased and the follicles were usually either small or invisible. In two cases the follicles appeared as white, pin-point areas inclosed by dark-red, pin-head-sized areas, which in turn were surrounded by the lighter red splenic parenchyma. Small, punctiform hæmorrhages occurred beneath the capsule in one instance and scattered through the substance of the spleen in others. The trabeculæ were prominent in only one instance in which the age of the subject was apparently between 50 and 60 years. In one case a reddish-white infarct 4 millimeters in its greatest width was encountered.

Histological examination of the spleen.—The chief lesion in the spleen is found in a marked congestion and hyperplasia of the pulp tissue with small hæmorrhages occurring beneath the capsule and throughout the pulp in a very large percentage of cases. The degree of the congestion varies. It is frequently especially marked at the immediate periphery of the lymphoid follicles. These lymphoid follicles are for the most part both relatively and absolutely small and seldom show any signs of proliferation. The bacteria in the follicles are very scarce. Some cases show fairly large areas of necrosis of the pulp in the areas of the hæmorrhage. The swelling of the endothelial cells of the lymph sinuses is by no means an infrequent occurrence, although evidence of their multiplication is not seen.

Kidneys.—Punctiform hæmorrhages measuring several millimeters in diameter were frequently observed in the capsules of the kidneys, which usually stripped easily from the surfaces of the organs. The kidneys were usually rich in blood, and in a number of instances after the removal of the capsule a red, granite-like appearance was observed due to the deeply injected vessels in contrast to the yellowish parenchyma of the organ. The stellate veins were usually deeply injected. Small hæmorrhages about the surface vessels of the kidneys were unusual, but were observed in three cases. On cut section either parenchymatous or early fatty changes were almost invariably evident. The glomeruli were frequently swollen and often appeared as fine, reddish, pin-point-sized areas. Petechiæ were frequently seen in the pelves and upper portion of the ureters.

Histological examination of the kidneys.—Extreme degeneration of the parenchyma of the kidney is a constant feature. The degeneration is in the form of an extreme cloudy swelling and

granular degeneration which is especially marked in the cells of the convoluted tubules but also involves the epithelium covering the glomerular tufts. It is not infrequent for this degeneration to have proceeded so far as to constitute a necrosis. The most striking changes in the glomeruli consist in the degeneration of the epithelium of the tufts which has already been referred to. Intense congestion of the glomerular vessels is practically always present and, in a few cases, a small amount of fluid exudate into Bowman's capsule is found. In no case was the leucocytic exudate into Bowman's capsule excessive. No evidence of proliferation of the cells lining Bowman's capsule was seen. In two cases of the series, fibrin thrombi as described by Herzog [11] constituted a very prominent feature in the sections of the glomeruli. Œdema of the kidney is evident in the sections, and very numerous, small hæmorrhages which were chiefly situated beneath the capsule were often seen. Some of the cases were the seat of an old chronic interstitial process on which the changes above indicated had been superimposed. The finding of casts in the tubules was rare, but the presence of a coagulated fluid exudate or transudate was not at all infrequent. Sections of the mucosa of the pelvis of the kidney were not obtained.

Liver.—The liver also invariably showed either cloudy swelling or early fatty degeneration. A few small hæmorrhages about 2 to 3 millimeters in diameter situated beneath the capsule were observed in two cases, in one of which the hæmorrhages were also linear in character, measuring as much as one-half centimeter in length and about 1 or 2 millimeters in width. Small metastatic abscesses such as are occasionally observed in bubonic plague were not encountered in either the liver or the kidney.

Histological examination of the liver.—The sections show practically always an extensive congestion, and cloudy swelling is a constant feature. While no large areas of necrosis are found, many of the specimens show small areas where one or two or three cells at one place have undergone necrosis. Extensive fatty changes were not noted, although selective stains for fat were impossible. Hæmorrhages beneath the capsule occurred, but hæmorrhages in the substance of the organ were scarcely ever noted and were never of any extent. In some places the liver cells were considerably compressed by the engorged vessels. In some of the cases rather extensive bile-stasis was manifest by the abundance of biliary pigment contained within the liver cells.

[11] *Pub. Bur. Govt. Labs.* (1904), No. 23, 9.

The *adrenals* sometimes showed congestion. No pathological changes were observed in the *pancreas, thyroid,* or *thymus gland,* though the tissues about the latter were usually markedly œdematous. In the *uterus,* hæmorrhages were frequently observed; in the other *sexual organs* or *bladder,* no special changes were noted. The central nervous system in the instances we had occasion to examine showed no gross anatomical changes with the exception of hyperæmia and sometimes œdema of the meninges.

Bacteriology.—Microscopical preparations and cultures were made from the organs in every case. In each instance the pest bacillus was present in the blood. The bacilli were always much more numerous in the lungs and in the bronchial lymphatic glands at the bifurcation of the trachea than in any of the other organs or in the blood. In the lungs they were found frequently packed together in great masses and were usually present in pure culture. In but two instances were diplococci encountered in small numbers. The plague bacilli were always more numerous in the spleen than in the blood. In no other disease are such enormous masses of bacteria encountered in the lung. In the tonsil, with but one exception, the number of plague bacilli found was small, usually not more than was observed in the blood. Staphylococci and streptococci and even Gram-positive bacilli were seen in preparations from the tonsils in several cases. In scrapings from the mucosa of the bronchi, plague bacilli were often abundant, but not always so.

Conclusions.—From the study of the human lesions and those produced experimentally in animals,[12] it would appear that epidemic plague pneumonia results from inhalation, the primary point of infection being the bronchi. Along the bronchioles the infection extends by continuity directly into the infundibulum and air cells, or by contiguity through the walls of the bronchioles to the contiguous tissue of the lung, and gives rise to a consecutive peribronchial inflammation in the tissues immediately surrounding the bronchioles. From these areas the infection rapidly spreads to the adjacent pulmonary tissue and visceral pleura. The bacilli rapidly multiply and produce at first pneumonic changes of the lobular type, and shortly afterwards from the fusion of several rapidly spreading areas more general lobar involvement of the lung tissue. The blood becomes quickly infected, and a true bacteræmia results in every case. Secondary pathological changes occur, particularly in the spleen, bronchial glands, heart, blood vessels, kidneys, and liver. The

[12] See IV, p. 173 of this report.

fact that the bronchial glands at the bifurcation of the trachea are always much more severely affected than any of the other lymphatic glands argues against the theory that epidemic pneumonic plague is primarily a septicæmic disease, and that the lungs are infected secondarily from the blood. Moreover, in the earliest stage of the disease, the blood may be free from plague bacilli.[13] The conditions observed in the trachea and bronchi in epidemic pneumonic plague, together with the character of the pulmonary exudate, is pathognomonic of this condition. From the appearance of the mucous membranes of the throat, larynx, and trachea, a diagnosis of pneumonic plague may sometimes be suggested. The tonsils may become secondarily infected in pneumonic plague, just as other lymphatic glands—for example, the bronchial ones—become so infected. However, in pneumonic plague, death occurs before any very marked macroscopic changes occur in the tonsils. There is no doubt also that the tonsils may become primarily infected in epidemics of pneumonic plague, just as has occurred in sporadic cases during epidemics of bubonic plague. This, however, is not the common channel of primary infection, and in such cases involvement of the lymphatic glands of the neck occurs early in the course of the disease. The fact that the œsophagus was found to be normal in every case examined and that the intestines showed only slight lesions constitutes another argument against the idea of the occurrence of primary intestinal plague infection in man, since in many of the pneumonic cases plague bacilli must have been repeatedly swallowed in the bronchial secretions and in the saliva.

[13] See p. 202 of this report.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

VIII. SUSCEPTIBILITY OF ANIMALS TO PNEUMONIC PLAGUE.

By RICHARD P. STRONG AND OSCAR TEAGUE.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Many cultures isolated by us during the Manchurian epidemic from the lungs at necropsy have demonstrated the same pathogenicity for animals as virulent bubonic strains of the plague bacillus. The pneumonic cultures have shown themselves to be particularly pathogenic for mice, rats, guinea pigs, and monkeys (*Cynomolgus philippinensis* Geoff.), these animals dying from the same doses and succumbing within the same period after inoculation as has been observed after infection with bubonic strains. Some evidence was introduced at the Conference held at Mukden that suggested that when the pneumonic strains were injected subcutaneously into the guinea pigs, usually septicæmia was produced very quickly and typical buboes were not obtained. Moreover, it was affirmed that the guinea pigs died within a shorter time after inoculation than in the cases in which bubonic strains were employed. However, in these instances it appears that the results were dependent upon the size of the dose inoculated, as much as one-half of an agar-culture having been employed in the infection. We showed in Mukden that the cutaneous or subcutaneous inoculation of very small doses of the pneumonic strain into guinea pigs gave rise to the typical lesions observed in these animals after inoculation with virulent bubonic strains, particularly to typical buboes, to miliary abscesses in the spleen, and to secondary septicæmia with hæmorrhages in the different organs. Our statements at the Conference in this respect have since been borne out by extensive experiments performed by us in Manila and it has been conclusively shown, in addition, that when guinea pigs are inoculated with the pneumonic cultures by inhalation, they develop primary infection of the glands of the

neck, with secondary septicæmia and occasionally secondary pneumonia or, in some cases, primary pneumonia with secondary septicæmia. Very rarely does the spleen show miliary abscesses in such cases, the animals dying before such lesions develop.

In *monkeys* (*Cynomolgus philippinensis* Geoff.), also, the cutaneous or subcutaneous injection of the pneumonic cultures causes typical bubonic infection. Monkeys infected by the same cultures by inhalation develop primary pneumonic plague with secondary septicæmia and without involvement of the glands of the neck.

Tarbagans.—There has been considerable evidence brought forward during the past in support of the view that plague has existed in epizoötic form among a species of marmot, the tarbagan (*Arctomys bobac* Schreb.).[1] (See Plate VI.) However, there has been no direct bacteriological proof of this fact, and we have known nothing definite before in regard to the susceptibility of this animal to plague infection, though, according to Preble, *loc. cit.*, Tchaoushow showed these animals were susceptible to plague infection. Our own experiments on tarbagans were carried out in Mukden where, by the kindness of the Hon. Alfred Sze, imperial commissioner to the Plague Conference, we were supplied with these animals for experimental purposes. From our experiments we were able to show for the first time that cutaneous or subcutaneous infection of the tarbagan with virulent cultures of the pneumonic strain gives rise in these animals either to an acute bubonic or to subacute and chronic forms of plague infection. In some instances we have shown by comparative experiments that the tarbagan seems as equally susceptible to cutaneous or subcutaneous infection as the guinea pig, these animals dying in about the same time (two and one-half to five days after infection) and from the same doses of the organism. In these instances there are hæmorrhages about the point of inoculation, typical buboes, and swelling of the spleen. In other instances, after infection with the same organism and with the same doses, the tarbagans may suffer from subacute and chronic forms of plague infection. In three of these animals killed by chloroform from ten days to two weeks after infection,

[1] For evidence of this fact, see Report of the International Plague Conference *under* Tarbagans. *Also*, The Tarbagan and Plague. By Paul Preble. Reprint from the United States Public Health Reports (1912), No. 68. This latter article entirely omits our own experiments on tarbagans while giving other observations on these animals reported at the International Plague Conference.

there were found at necropsy abscesses measuring several millimeters in diameter in the subcutaneous tissues or in the abdominal muscles, near the point of inoculation, and swelling of the inguinal glands, while the liver and spleen showed indurated, yellowish nodules also measuring several millimeters in diameter. (See Plate XII, fig. 2.) Plague bacilli were present in small numbers in the abscesses and in the nodules in the spleen and liver. These animals, judging from their condition at the time they were killed, would probably have lived at least several weeks longer. The lesions present were similar to those which have been described in rats which have succumbed to chronic plague infection. We have also shown that the tarbagan is also susceptible to primary pneumonic plague when infection has taken place by inhalation. Death then occurs three or four days after infection from primary pneumonia and secondary septicæmia. These experiments were performed with the species *Arctomys bobac* Schreb.[2] We also showed that another species of marmot (*Spermophilus citillus* Linn.), very common about Mukden and the vicinity, was susceptible to acute plague infection, these animals dying in from three to seven days after cutaneous or subcutaneous inoculation of small doses of the pneumonic strain and exhibiting at necropsy hæmorrhages about the point of inoculation, typical buboes, and acute, splenic tumor.[3]

DONKEYS.

Some evidence was introduced at the International Plague Conference to show that donkeys became infected with pneumonic plague during the epidemic. Dr. W. S. Yang reported to the Conference [4] the death of 10 donkeys, the first of which died with cough and expectoration of blood. In the case of one of these animals, a necropsy was performed and cultures were made from the heart, spleen, lungs, and liver. All of these cultures were said to show plague bacilli. It was also announced that Doctor Otsuki in Fushun had observed at necropsy 2 donkeys in which there was hepatization of the lungs, in one in the right and in the other in the left caudal lobe. The pathological

[2] Petrie has shown (Report of the International Plague Conference, p. 235) that *Arctomys bobac* Schreb. found in Manchuria may be infected with the flea, *Ceratophyllus silantievi* Wagner, and that this flea will bite man. Tiraboschi and D-Kolbasenko have also described fleas on the tarbagan in Russia.

[3] For the details of these experiments, see Report of the International Plague Conference, pp. 237 and 385.

[4] *Ibid.*, p. 440.

changes in the lungs were said to be similar to those seen in the cases of human infection. In regard to the question of plague infection in donkeys, the Conference resolved that the question of the occurrence of pneumonic plague in these animals should be made the subject of a special study with regard to their liability to the infection. We, accordingly, have attempted to infect donkeys experimentally with pneumonic plague by spraying suspensions of virulent strains of pneumonic-plague bacilli into a closed canvas bag, fastened about the donkey's head in such a manner that it was necessary for the animal to inhale the bacteria in breathing. The experiments were performed as follows:

Experiment 1.—September 16. Two 48-hour agar-slant-cultures of a virulent pneumonic strain of the plague bacillus (isolated a few days previously from monkey No. 5635, which died of pneumonic plague) were suspended in saline solution and two-fifths of this suspension sprayed into the sacks surrounding the head of each of 2 donkeys. One of the donkeys coughed several times while the spraying was continued. The time of the spraying occupied from three to four minutes. The remaining quantity of the suspension of the agar-cultures, used in attempting to infect the donkeys, was sprayed into a closed glass cage containing 6 guinea pigs, and 5 loops of the same suspension were rubbed over the shaved abdomen of another guinea pig. All of these guinea pigs died of plague infection, the first six either of pneumonia or septicæmia with involvement of the cervical glands and the seventh guinea pig of bubonic infection. Both of the donkeys remained entirely healthy.

Experiment 2.—September 29. One 48-hour agar-culture of a virulent pneumonic strain of *Bacillus pestis*, isolated a few days before from animal No. 5741, which died of plague infection, was suspended in about 10 cubic centimeters of salt solution and about two-thirds of this suspension sprayed into both nostrils of a third donkey. The remainder of the suspension of this culture was then sprayed into a closed glass cage, containing 6 control monkeys (Nos. 5771 to 5776). All of the monkeys died later of pneumonic-plague infection. The third donkey remained healthy.

Experiment 3.—October 7. A large pneumonic area of the left lower lobe of a monkey that had just succumbed to pneumonic-plague infection was cut into small pieces and crushed with a pair of forceps in salt solution and the lung thoroughly broken to pieces. The suspension amounted in volume to about 20 cubic centimeters. One-half of this suspension was sprayed into a canvas sack surrounding the nostrils and head of one donkey and nearly all of the other half into a second sack over the nostrils and head of a second donkey. Guinea pig (No. 5802) was also inoculated cutaneously with 5 *œsen* of this same suspension. The guinea pig died three days later with typical bubonic-plague infection. The two donkeys remained entirely healthy.

Therefore, although we never failed to infect guinea pigs and monkeys with pneumonic plague by the same cultures which were sprayed into the nostrils of the donkeys, we were entirely

unable to infect the donkeys, even when they were made to inhale air charged with the most virulent cultures of pneumonic strains of the plague bacillus for a period of as long as five minutes at a time. We, therefore, do not consider donkeys susceptible to pneumonic-plague infection, and these experiments render it doubtful that these animals played any part in the dissemination of pneumonic plague during the Manchurian epidemic, and suggest that in the reported cases of pneumonic plague in donkeys the infecting organism was not *Bacillus pestis*, but, perhaps, some other organism of the hæmorrhagic septicæmia group.

DOGS.

At the Mukden Conference,[5] 1 case of pneumonic-plague infection in a dog, observed by Doctor Takami, was referred to in which there was pneumonia in the caudal lobe of the left lung. This dog was found in a house where 7 people had died of plague infection. The Conference also resolved that the question of the occurrence of pneumonic plague in dogs should be made the subject of special study with regard to their liability to this infection. Accordingly, we also performed experiments with this object in view. The results were as follows:

On November 4, 2 fully grown dogs were placed in a closed glass cage and a suspension of two 48-hour agar-cultures of a virulent pneumonic strain of the plague bacillus was sprayed into the cage for two periods of two and one-half minutes, each after a brief interval between them. The first dog, No. 5880, died on November 9, five days after infection. The necropsy showed there was pneumonia of both lungs. In the right lung all the lobes were involved. Only a small portion at the apex of the upper lobe did not show pneumonia. In the left lung, both lobes, with the exception of the apex of the upper lobe, were also involved. The pneumonia was in the stage of engorgement with the exception of small bronchial areas scattered throughout the lung, measuring from about 2 millimeters to 1 centimeter in diameter. These areas of bronchial pneumonia were grayish in color on the surface of the lung, and on section they were grayish at the periphery and in the center red and slightly granular. The areas were not wedge-shaped, but were circular in outline (see Plate XII, fig. 1). Smears from the lungs showed comparatively few plague bacilli and a few streptococci. The large bronchi were not reddened. There was much mucus in the

[5] *Loc. cit.*

trachea, but the mucous membrane here was also not reddened. The cervical glands appeared normal. There was no œdema of the cervical tissues. The spleen was swollen, but contained no miliary abscesses. The liver showed cloudy swelling, and also contained no miliary abscesses. Microscopical preparations from the spleen showed a few plague bacilli. Cultures from the heart and lung developed numerous colonies of the plague bacillus.

The other dog (No. 5881) died March 21, seventeen days after infection. He was considerably emaciated. The necropsy showed that the lymphatic glands were nowhere swollen. There were no hæmorrhages or œdema in the tissues about the neck. The trachea and larger bronchi contained frothy, reddish mucus. The left lung was normal throughout. The upper lobe of the right showed advanced hepatization throughout and sank when placed in water. Two grayish wedge-shaped infarcts, measuring from 1 to 1.5 centimeters at the base, were present in this lobe. The whole lobe showed reddish-gray hepatization with beginning resolution. The middle and lower lobes were somewhat congested, but contained no pneumonic areas. Microscopical preparations from the lung showed fair numbers of *Bacillus pestis*. No other organism was present in the lung, as was demonstrated by cultures. Microscopical preparations from the spleen showed a few bipolar forms and a number of involution forms of the plague bacillus.

Therefore, our experiments upon dogs show that these animals are only moderately susceptible to pneumonic plague but that, when exposed to severe infection, they may contract primary pneumonic plague and die of the disease.

Shibayama [6] showed that dogs were not very susceptible to subcutaneous infection with the pneumonic strain, but that they sometimes succumbed from the subcutaneous inoculation of large doses or from intraperitoneal inoculation.

Pigs.—It was stated that over 300 pigs had died during the epidemic at Harbin, but there was no evidence presented which showed that the disease from which they succumbed was bubonic or pneumonic-plague infection, nor was any evidence submitted which showed that the disease was not hog cholera or swine plague.

[6] *Ibid.*, p. 46.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

IX. PROTECTIVE INOCULATION AGAINST PNEUMONIC PLAGUE.

By RICHARD P. STRONG AND OSCAR TEAGUE.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

The epidemic of pneumonic plague, which raged in Manchuria and northern China during the winter of the year 1910–1911 and which caused the death of over 50,000 people, brought before us, among other problems, one of particular importance, namely, that of protective inoculation against the disease.

The Chinese Government spent over 100,000 dollars (Mexican) on plague prophylactics during the epidemic, but, at its close, their efficacy was doubted.

Inoculations with killed cultures were alone employed during the epidemic. One hundred and thirty-two people were inoculated at Harbin; 22 of these contracted plague, 13 after one injection, 8 after two injections, and 1 after three injections. Of the 8 who fell sick after two injections, 2 contracted plague six days, 2 ten days, 2 fourteen days, 1 twelve, and 1 twenty-seven days after the inoculation. Of the 13 who contracted plague after one injection, 12 contracted plague after two weeks and one after six days.

Unfortunately, it was not possible to ascertain how many of those inoculated were afterward exposed to pneumonic-plague infection or how many, when exposed, protected themselves by the wearing of masks. Dr. Wu [1] reported to the Conference the case of Mr. Liu, a medical student who worked with pneumonic-plague patients for a whole month with no other precaution than masking. On January 2 he was inoculated, and eight days after he contracted pneumonic plague and died. He also reported the case of Dr. Hsu who was inoculated on January 4 and contracted pneumonic plague on January 22, and stated in addition

[1] Report of the International Plague Conference held at Mukden, April, 1911. Manila (1912), 332.

that of 20 other individuals who were inoculated at the same time none became infected.

At Fuchiatien, 439 individuals were inoculated with Haffkine's vaccine and with antiplague serum. Sixteen individuals received three inoculations, two of Haffkine's vaccine and one of serum. None of these became infected. Thirty individuals received two injections, either with Haffkine's vaccine or with Haffkine's vaccine and serum. None of these, also, became infected. Of 393 individuals who were vaccinated once with Haffkine's vaccine, 4 died of plague, 1 eight days, 1 ten days, 1 eighteen days, and 1 thirty-two days after inoculation. The same comment applies to these statistics, namely, that we have no evidence as to how many of the 439 individuals who were inoculated were subsequently exposed to infection and how many protected themselves by the use of masks.

Approximately 14,000 individuals were inoculated with killed cultures of the plague bacillus, during the epidemic, but the great majority of these individuals were never exposed to plague infection. Therefore, we have no positive evidence as to what protection was conferred upon them by inoculation. The only definite conclusion which, it appears, we were justified in drawing from the statistics obtained from the epidemic is that prophylactic inoculations, by means of dead cultures, have sometimes been ineffective in preventing pneumonic-plague infection. Some individuals, inoculated twice, and some even three times, have contracted the disease.

From the evidence presented before the International Plague Conference, held in Mukden in April, 1911, it was resolved by the Conference that the statistics, which were collected during the epidemic, did not allow of any definite conclusion about the value of active prophylactic inoculation against pneumonic plague. Nevertheless, the Conference further resolved that, as the statistical evidence pointed to the conclusion that some degree of protection is conferred against bubonic plague by the use of prophylactics, therefore, there were *a priori* grounds for the use of protective inoculation against pneumonic plague.

The Conference, therefore, advised that experiments on the protective inoculation of animals should be carried on and the immunity of the animals tested by their exposure to infection to pneumonic plague by inhalation, in order to find out which prophylactic could be best used against pneumonic plague, and, if another outbreak of this disease should occur, that dead bacillary prophylactics, Lustig and Galeotti's nucleo-proteid, and Strong's method of vaccination, with a living attenuated culture,

should be tried in selected communities, under rigorous scientific conditions.

At the time the Conference was in session, there was no experimental evidence whatever as to the protection afforded by prophylactic inoculations against pneumonic plague, and no experiments of this nature in animals, other than the ones which will be discussed in this paper, hitherto have been reported. The reason for this appears to be obvious from the fact that there has been no other great epidemic of pneumonic plague within modern times, and the question of prophylactic inoculation in man, as a practical means of protection against this disease, during an epidemic, has not hitherto arisen. We, therefore, determined to investigate this question experimentally. The assumption that, because prophylactic inoculations furnish some degree of protection against bubonic plague they also would be protective against pneumonic-plague infection hardly would seem warranted, since the portal of entry of the infecting agent is so different in the two conditions. Moreover, the plague organism finds in the pulmonary tissues a much more favorable and extensive medium for its multiplication and diffusion than it does in the lymphatic glands. In bubonic plague, the lymphatic glands may be said to act as filters against the general invasion of the body by the plague bacillus, while in primary pneumonic plague there is no such mechanism for the defense of the host, the bacilli spreading rapidly throughout the lung and invading the circulation in every instance in a comparatively short time and apparently before the host has had time to produce any appreciable quantity of immune substances. The bronchial lymphatic glands in primary pneumonic plague offer resistance to the invasion of the plague bacillus, and in every case of this disease these glands are very acutely inflamed and frequently almost of a black color from the resulting toxic hæmorrhages in the glandular substance. However, by the time the bronchial glands have become involved, the bacteria have already spread so extensively throughout the lung substance that a bacteræmia has usually occurred.

The infection or immunity of the host, for certain bacteria, sometimes depends solely upon the portal of entry of the organism; for example, the same quantity of cholera vibrios, injected subcutaneously in man or administered by the mouth, may produce entirely different results. In the former instance, a local and general reaction is obtained, but the bacteria quickly die, while in the latter instance, the vibrios may pass to and multiply in the intestines and Asiatic cholera result.

As another example of the importance of the portal of entry of the organism in relation to infection and immunity may be cited the fact that the tetanus bacillus, which frequently resides normally as a harmless commensal in the intestine of the horse, when injected beneath the skin of this animal, may produce tetanus and death.

These examples serve to emphasize the importance of experimental work upon the subject of protective inoculation against pneumonic-plague infection, and show that it does not follow that, because there is evidence that protective inoculation is sometimes successful in the case of bubonic plague, it will necessarily also be efficient in the case of pneumonic plague.

In our experiments in immunization, we used both guinea pigs and monkeys. In selecting a method of prophylactic inoculation for the production of the immunity, we naturally chose the one of *vaccination;* that is, inoculation with a living attenuated organism, as this method unquestionably has been shown to produce a much higher immunity against bubonic-plague infection than any other in which killed cultures of the plague organism or its extracts are employed.

The accompanying Table I, taken from the previous experiments of one of us (Strong),[2] shows the comparative value of the different methods of immunization employed against cutaneous and subcutaneous plague infection in animals.

TABLE I.—*Combined table comparing efficiency of different methods of immunization.*

KILLED PEST CULTURES.

Kind of animal and series No.	Number inoculated.	Number dead before immunity tested.	Immunized.	
			Number.	Percentage.
Monkeys:				
Bouillon-cultures—				
Series 5	8		3	37
Series 49	9		2	22
Agar-cultures—				
Series 9	3			
Series 25	20	3	4	23
Series 48	15	1	4	28
Total	55	4	13	25
Guinea pigs (killed agar-cultures):				
Series 50 (total)	15		4	26

[2] *This Journal, Sec. B* (1907), **2**, 238.

TABLE I.—*Combined table comparing efficiency, etc.*—Continued.

LIVING PEST AVIRULENT STRAIN I.

Kind of animal and series No.	Number inoculated.	Number dead before immunity tested.	Immunized.	
			Number.	Percentage.
Monkeys:				
Series 4	6		5	83
Series 11	5		1	20
Series 12	8		4	50
Series 18	10		5	50
Series 51	15		8	53
Total	44		23	52
Guinea pigs:				
Series 32	11	5	5	83
Series 37	9	2	6	85
Series 39	15		8	53
Series 41	21		15	71
Series 46	15		12	80
Total	71	7	64	72

LIVING PEST AVIRULENT STRAIN II.

Monkeys:				
Series 17	4		3	75
Series 21	12		8	66⅔
Series 24	18	4	13	92
Series 52	15	1	7	50
Total	49	5	31	70
Guinea pigs:				
Series 33	10	3	7	100
Series 38	7	1	4	66⅔
Series 40	15	1	11	78
Series 47	15	1	14	100
Total	47	6	36	88

EXTRACTS OF PLAGUE BACILLUS (FREE RECEPTORS).

Monkeys:				
Series 7	4		1	25
Series 26	5		1	20
Total	9		2	22

ARTIFICIAL AGGRESSIN.

Monkeys:				
Series 29 (total)	32		4	12½
Guinea pigs:				
Series 29	2		0	0
Series 30	2		0	0
Series 31	6		1	16⅔
Series 36	12		0	0
Series 42	4		2	50
Total	26		3	11

TABLE I.—*Combined table comparing efficiency, etc.*—Continued.

NATURAL AGGRESSIN.

Kind of animal and series No.	Number inoculated.	Number dead before immunity tested.	Immunized.	
			Number.	Percentage.
Guinea pigs:				
Series 35	15		4	26
Series 44	12		4	33⅓
Total	27		8	30

KLEIN'S METHOD.

Guinea pigs:				
Series 54	6		2	33⅓
Series 56	7		2	28
Total	13		2	30

It is demonstrated that in the case of guinea pigs, vaccinated with an avirulent culture, about 80 per cent are protected against a severe cutaneous plague infection, and in monkeys, vaccinated in the same way, about 61 per cent are so protected against subcutaneous infection.

It was possible to immunize against the same severe cutaneous test but 26 per cent of the guinea pigs with killed cultures. Kolle and Otto, in numerous previous experiments on guinea pigs, were never able to immunize more than 10 per cent of these animals by means of repeated inoculations of killed cultures. It will be noted also in the accompanying table that in the experiments in which living attenuated cultures were used for the immunization of guinea pigs, in one series 72 per cent and in another 88 per cent were protected against cutaneous infection. These experiments are confirmatory of the statement that the living attenuated culture gives a much higher degree of immunity against cutaneous plague infection than a killed one.

In order to retest the value for immunization against cutaneous infection of the culture used for the vaccination in the experiments recorded in this paper, the following experiment was performed:

EXPERIMENT NO. 1.

Twenty-four guinea pigs each received subcutaneously on June 9, 1911, one 48-hour agar-slant-culture of living avirulent plague. One died (intraperitoneal inoculation) in less than twenty-four hours after vaccination and the others survived. Two weeks later, 11 of these vaccinated guinea pigs were subjected to infection with virulent plague bacilli by cutaneous

inoculation in order to retest the value for immunization against cutaneous infection of the culture used for the vaccination in the experiments recorded in this paper. The 12 remaining vaccinated guinea pigs were subjected to infection by inhalation on the same day. The virulent culture used was isolated from our human case (No. 9) of pneumonic plague at Mukden. The culture was passed through a guinea pig and a monkey, and fresh cultures on agar from the monkey's blood were employed in the experiment.

The 11 vaccinated guinea pigs, together with 12 normal guinea pigs as controls, were subjected to cutaneous inoculation in the following manner: The growth of one agar slant of the virulent culture just described was suspended in 5 cubic centimeters of peptone solution and 5 *œsen* of this suspension were rubbed over the shaved and scarified abdomen of each of the guinea pigs. The result was as follows:

Cutaneous infection.

Vaccinated guinea pigs.[a]	Normal guinea pigs.[b]
No. 5281 survived.	No. 5360 died in 5 days.
No. 5282 died in 13 days.	No. 5361 died in 10 days.
No. 5283 survived.	No. 5362 died in 3 days.
No. 5284 survived.	No. 5363 died in 10 days.
No. 6285 died in 14 days.	No. 5364 died in 8 days.
No. 5286 died in 7 days.	No. 5365 died in 6 days.
No. 5287 died in 6 days.	No. 5366 died in 9 days.
No. 5288 survived.	No. 5367 died in 4 days.
No. 5289 died in 7 days.	No. 5368 died in 6 days.
No. 5290 survived.	No. 5369 died in 5 days.
No. 5291 survived.	No. 5370 died in 3 days.
	No. 5371 died in 6 days.

[a] Total: 6 survived; 5 died.
[b] Total: 0 survived; 12 died.

Fifty-five per cent of the vaccinated guinea pigs and none of the controls survived. This experiment merely demonstrated the immunizing value of the culture against cutaneous or bubonic infection. It is desired to emphasize again the fact that guinea pigs can usually *only* be successfully immunized against cutaneous or bubonic infection by means of a living attenuated culture. Only a very few of the animals inoculated with killed cultures, when exposed to infection, survive.

For the purpose of testing the immunity of the remaining 12 vaccinated guinea pigs against pneumonic infection, the growth of two agar slants of the virulent culture described above was suspended in 30 cubic centimeters of normal saline solution. This was placed in a glass receiver of an ordinary nasal spray. Air was supplied by a hand-force-pump and rubber tubing connection. The suspension could, in this manner, be sprayed in a fine vapor for a distance of several feet. Six vaccinated and six normal

guinea pigs were placed together in a closed glass cage about .75 meter square, the nozzle of the spray introduced at one side of the cage, and the suspension sprayed into the cage for about one minute. The position of the nozzle was then changed to the opposite side of the cage, and the suspension then sprayed for another minute. The animals remained in the closed cage about ten minutes after the spraying was discontinued. The other 6 vaccinated guinea pigs with 6 controls were sprayed in like manner in a similar cage. While performing all of the spraying experiments referred to in this article, we always wore masks[3] and goggles, such as we employed when working with pneumonic-plague patients during the recent epidemic in Manchuria. Rubber gloves were also worn in handling the animals.

The result in this first series of animals was as follows:

SERIES I.—*Infection by inhalation.*

Vaccinated guinea pigs.[a]	Normal guinea pigs.[b]
No. 5268 died in 6 days.	No. 5348 died in 4 days.
No. 5269 died in 6 days.	No. 5349 died in 4 days.
No. 5270 survived.	No. 5350 died in 4 days.
No. 5273 survived.	No. 5351 died in 5 days.
No. 5274 survived.	No. 5352 died in 11 days.
No. 5275 survived.	No. 5353 died in 13 days.
No. 5276 survived.	No. 5354 died in 4 days.
No. 5277 survived.	No. 5355 died in 3 days.
No. 5278 died in 6 days.	No. 5356 died in 4 days.
No. 5279 survived.	No. 5357 died in 4 days.
No. 5280 survived.	No. 5358 died in 3 days.
	No. 5359 died in 3 days.

[a] Total: 8 survived; 3 died.
[b] Total: 0 survived; 12 died.

In this series 72.7 per cent of the vaccinated animals survived, while all of the unvaccinated control ones died of plague infection.

EXPERIMENT NO. 2.

On August 10, 1911, each of 24 guinea pigs of a second series was vaccinated with one agar slant of a 48-hour culture of avirulent plague and 23 survived the treatment; one died of an injury. Four weeks later, these vaccinated guinea pigs, together with 24 controls, were subjected to infection by inhalation with a virulent strain of plague. The culture used was originally obtained from a pneumonic-plague necropsy at Muk-

[3] The experiments of Barber and Teague, see XII, p. 255 of this report, demonstrate that this mask (the Mukden type) was not a safe protection while carrying on these inhalation experiments. Fortunately, we escaped pneumonic-plague infection, probably because the spray was not directed toward us but into the cage.

den. After passage through a number of guinea pigs, a monkey was infected with the strain by inhalation. The monkey died of pneumonic plague, and a guinea pig was inoculated cutaneously with a portion of the pneumonic lung of the monkey. The culture sprayed was obtained from the blood of this guinea pig. The growth of two agar-slant-cultures was suspended in about 40 cubic centimeters of normal saline solution and about two-thirds of the suspension was sprayed into the cages as in the preceding experiment. The result was as follows:

SERIES II.—*Infection by inhalation.*

Vaccinated guinea pigs.[a]	Normal guinea pigs.[b]
No. 5568 died in 4 days.	No. 5617 died in 8 days.
No. 5569 survived.	No. 5618 died in 4 days.
No. 5570 died in 4 days.	No. 5619 died in 4 days.
No. 5571 survived.	No. 5620 died in 3 days.
No. 5572 died in 3 days.	No. 5621 died in 4 days.
No. 5573 survived.	No. 5622 died in 3 days.
No. 5574 died in 10 days.	No. 5623 died in 4 days.
No. 5575 survived.	No. 5624 died in 3 days.
No. 5576 survived.	No. 5625 died in 6 days.
No. 5578 survived.	No. 5626 died in 4 days.
No. 5579 survived.	No. 5627 died in 4 days.
No. 5580 survived.	No. 5628 died in 3 days.
No. 5581 survived.	No. 5629 survived.
No. 5582 survived.	No. 5630 died in 3 days.
No. 5583 survived.	No. 5631 died in 3 days.
No. 5584 survived.	No. 5632 died in 4 days.
No. 5585 survived.	No. 5633 died in 5 days.
No. 5586 survived.	No. 5634 died in 4 days.
No. 5587 survived.	No. 5635 died in 3 days.
No. 5588 died in 7 days.	No. 5636 died in 3 days.
No. 5589 survived.	No. 5637 died in 3 days.
No. 5590 died in 5 days.	No. 5638 died in 4 days.
No. 5591 died in 7 days.	No. 5639 died in 4 days.
	No. 5640 died in 3 days.

[a] Total: 16 survived; 7 died.
[b] Total: 1 survived; 23 died.

Sixteen, or 69.6 per cent, of the vaccinated animals survived, while all but one of the control ones (that is, 4.1 per cent) died of plague infection. This one control animal probably screened itself in some way behind the other guinea pigs and thus avoided infection.

Summary of all inhalation experiments upon guinea pigs.

Guinea pigs.	Total.	Survived.	Percentage of survivals.
Vaccinated	34	24	70.6
Normal	36	1	2.8

Therefore, from these two series of experiments, we see that 70.6 per cent of the vaccinated animals proved immune when exposed to infection by means of spraying virulent plague bacilli into the air which they breathed, and hence were immune to this method of infection. However, it is necessary to examine closely into the nature of this immunity.

It is seen that of *36 control unvaccinated* guinea pigs, all except one succumbed to the infection induced by spraying. Upon post-mortem examination, the following changes, which applied to practically all of the *control* animals, were encountered. In general, there were marked evidences of plague infection about the tissues of the neck and throat. The subcutaneous tissues showed extensive œdema, and there was swelling of the lymphatic glands of the neck and of those about the trachea. Usually the glands were not only swollen but more or less hæmorrhagic, and had the appearance of small buboes. Throughout the body, marked evidence of septicæmia was usually present. There were frequently extensive hæmorrhages in the intestinal wall. The spleen sometimes showed the typical changes encountered in plague infection with miliary abscesses. Pneumonia was present in only about 23 per cent of the control animals. These changes suggest that the primary point of infection evidently was located in the mucous membranes of the throat and that it did not usually occur in the bronchi or alveoli of the lung. From these lesions it would appear that normal guinea pigs, under the conditions of the experiment in which the spraying was carried on, do not usually develop primary plague pneumonia, but that infection occurs through the mucous membranes of the mouth and throat and infection and sometimes buboes of the glands of the neck and septicæmia result. It would appear that in guinea pigs, either on account of too shallow respirations or the small size of the larynx and trachea, the bacteria are not so likely to penetrate to the smaller bronchi by means of the inspired air. Instead, they are apparently arrested by the mucous membrane of the throat. Attention must be called to the fact that the spray employed was not so fine a one as that used in the subsequent experiments on monkeys and that the bacteria were, therefore, sprayed in larger particles in the first two series of experiments. Whatever the reason, however, the fact remains that primary pneumonic infection in the guinea pigs did not usually result. Therefore, the conclusions that can be drawn regarding the vaccinated guinea pigs

are that 70 per cent of these animals appeared to be immune against plague infection entering through the mucous membranes of the mouth and throat, but one can not conclude that this same percentage of animals would have proved to be immune if the organism really had been introduced directly into the lung by the bronchi.

Let us consider the experiments in relation to monkeys which give us much clearer information regarding immunization against pneumonic plague. Three series of experiments were performed as follows:

EXPERIMENT NO. 3.

Eleven [1] monkeys were vaccinated on June 23, 1911, each with one 48-hour agar-culture of avirulent plague. They were subjected to infection by inhalation as follows: Two vaccinated monkeys with two controls on July 7; 5 vaccinated ones with 5 controls on July 12; 5 vaccinated ones with 6 controls on July 14. The general mode of procedure was the same as in the preceding experiments. The result was as follows:

SERIES III.—*Infection by inhalation.*

Vaccinated monkeys.[a]	Normal monkeys.[b]
No. 5404 died in 5 days.	No. 5437 died in 4 days.
No. 5386 died in 5 days.	No. 5438 died in 4 days.
No. 5384 died in 6 days.	No. 5439 died in 5 days.
No. 5394 died in 5 days.	No. 5440 died in 5 days.
No. 5393 died in 5 days.	No. 5501 died in 5 days.
No. 5400 died in 4 days.	No. 5424 died in 3 days.
No. 5401 survived.	No. 5425 died in 3 days.
No. 5387 died in 3 days.	No. 5491 died in 4 days.
No. 5389 died in 5 days.	No. 5492 died in 3 days.
No. 5403 died in 5 days.	No. 5493 died in 4 days.
No. 5390 died in 4 days.	No. 5494 died in 3 days.
	No. 5495 died in 3 days.

[a] Total: Survived, 1; died, 10.
[b] Total: Survived, 0; died, 12.

Only one of the vaccinated monkeys, or 9 per cent, survived, while all of the controls died of pneumonic infection.

EXPERIMENT NO. 4.

On September 15, 1911, twenty-two monkeys were vaccinated each with one 48-hour agar-culture of living avirulent plague bacilli. Two weeks later they, together with 22 unvaccinated monkeys, were subjected to

[1] Additional monkeys were vaccinated in this and the following series but died in a cachectic condition before the date for testing their immunity arrived. In no instance were we able to show that they died of plague.

infection by inhalation with a pneumonic strain which had been passed through a series of guinea pigs. The growth from 3 agar-slant-cultures was suspended in about 40 cubic centimeters of normal saline solution and all of this suspension was used for the spraying. Only three or four vaccinated monkeys with the same number of controls were placed in a cage at a time during the spraying. They were then, of course, placed in separate cages. The result of the experiment was as follows:

SERIES IV.—*Infection by inhalation.*

Vaccinated monkeys.[a]	Normal monkeys.[b]
No. 5704 survived.	No. 5771 survived.
No. 5706 died in 6 days.	No. 5772 died in 5 days.
No. 5707 survived.	No. 5773 died in 5 days.
No. 5708 died in 4 days.	No. 5774 died in 6 days.
No. 5709 died in 6 days.	No. 5775 survived.
No. 5710 died in 5 days.	No. 5776 died in 5 days.
No. 5711 died in 5 days.	No. 5777 died in 5 days.
No. 5712 survived.	No. 5778 died in 6 days.
No. 5713 died in 7 days.	No. 5779 died in 6 days.
No. 5714 died in 8 days.	No. 5780 died in 5 days.
No. 5715 survived.	No. 5781 died in 5 days.
No. 5716 died in 6 days.	No. 5782 died in 6 days.
No. 5717 died in 5 days.	No. 5783 died in 8 days.
No. 5718 died in 6 days.	No. 5784 survived.
No. 5719 died in 8 days.	No. 5785 died in 6 days.
No. 5720 survived.	No. 5786 died in 4 days.
No. 5721 survived.	No. 5787 died in 4 days.
No. 5722 survived.	No. 5788 died in 5 days.
No. 5724 survived.	No. 5789 died in 4 days.
No. 5725 died in 6 days.	No. 5790 died in 5 days.
No. 5726 died in 6 days.	No. 5791 survived.
No. 5727 survived.	No. 5792 died in 5 days.

[a] Total: Survived, 9; died, 13.
[b] Total: Survived, 4; died, 18.

Nine, or 40.5 per cent, of the vaccinated monkeys survived, while 4, or 18.1 per cent, of the unvaccinated control ones also survived. In this experiment, evidently the method of producing the infection was not satisfactory, since four of the control unvaccinated monkeys did not develop plague infection. The results obtained in Series IV, therefore, do not give as valuable information as do the results obtained in Series III and V.

EXPERIMENT NO. 5.

On October 18, 1911, twenty-one monkeys were each vaccinated subcutaneously with one 48-hour agar-slant-culture of living avirulent plague bacilli. On November 2, these animals and 21 control unvaccinated ones were exposed to infection by inhalation in the same manner as in the preceding experiments. The result may be tabulated as follows:

SERIES V.—*Infection by inhalation.*

Vaccinated monkeys.[a]	Normal monkeys.[b]
No. 5818 died in 5 days.	No. 5854 died in 2 days.
No. 5819 died in 6 days.	No. 5855 died in 5 days.
No. 5820 died in 5 days.	No. 5856 died in 5 days.
No. 5822 survived.	No. 5857 died in 7 days.
No. 5823 died in 5 days.	No. 5858 died in 5 days.
No. 5824 died in 2 days.	No. 5859 died in 5 days.
No. 5825 died in 6 days.	No. 5860 died in 5 days.
No. 5826 died in 6 days.	No. 5861 died in 5 days.
No. 5827 died in 6 days.	No. 5862 died in 5 days.
No. 5828 died in 5 days.	No. 5863 died in 5 days.
No. 5829 died in 5 days.	No. 5864 died in 5 days.
No. 5830 died in 5 days.	No. 5865 died in 5 days.
No. 5831 died in 5 days.	No. 5866 died in 2 days.
No. 5832 died in 2 days.	No. 5867 died in 5 days.
No. 5833 died in 9 days.	No. 5868 died in 2 days.
No. 5834 survived.	No. 5869 died in 5 days.
No. 5835 died in 6 days.	No. 5870 died in 2 days.
No. 5837 died in 5 days.	No. 5871 died in 5 days.
No. 5838 died in 4 days.	No. 5872 died in 5 days.
No. 5839 died in 5 days.	No. 5873 died in 5 days.
No. 5840 died in 6 days.	No. 5874 died in 5 days.

[a] Total: Survived, 2; died, 19.
[b] Total: Survived, 0; died, 21.

Only 2, or 9.5 per cent, of the vaccinated animals survived, while all of the control monkeys died of pneumonic-plague infection.

Summary of all experiments upon monkeys.

Series.	Vaccinated monkeys.			Normal monkeys.		
	Survived.	Died.	Percentage of survivals.	Survived.	Died.	Percentage of survivals.
IV	9	13	41	4	18	18
V	2	19	9.5	0	21	0
III	1	10	9	0	12	0
Total	12	42	22	4	51	7.3

As we have done in the case of the unvaccinated guinea pigs, so it will be advisable in the case of the unvaccinated normal monkeys to examine into the character of the infection. The following observations apply to practically all of these control animals. At necropsy, there was absence of any sign of plague infection about the tissues of the neck. The submaxillary and cervical lymphatic glands and those about the trachea were

not swollen, nor was there any œdema of the cervical tissues, as was practically always seen in the control guinea pigs. In a number of cases, the tonsils were examined and found to be normal. There was frequently œdematous fluid in the trachea. The larynx and vocal cords were, as a rule, not injected. In a few cases the trachea was slightly reddened. There were not such marked evidences of a septicæmia as seen in the control guinea pigs. No hæmorrhages were noted in the intestines and omentum. The spleen and liver showed no miliary abscesses.

There were no cervical, axillary, nor inguinal buboes. *The lungs showed primary pneumonia in every case.* There was always much œdema of the lung. The pneumonia was either in the stage of engorgement or of red or early gray hepatization. In a number of the cases, a pleuritic exudate was observed over the hepatized areas. The plague bacilli were always most numerous in the lungs.

From these observations, it is obvious that the infection in monkeys occurred by inhalation and resulted in primary plague pneumonia. It also is evident that in some instances, in which monkeys are exposed to infection by inhalation, the primary point of infection may not only be the lungs but also the mucous membranes of the mouth and throat. That plague infection may occur through the mucous membranes of the mouth and throat in monkeys was demonstrated by placing a small quantity of plague bacilli, by means of a glass rod, on the posterior portion of the throat.[5]

These animals all died of plague septicæmia, with or without infection of the glands of the neck. That is, in the cases in which the infection was severe and the susceptibility of the animals more marked, the animals succumbed to septicæmia before cervical buboes developed. In none of these instances was pneumonia present. Primary plague pneumonia only results when infection by inhalation has in addition taken place.

Therefore, experiments performed with monkeys in the vaccination and subsequent infection of the animals by inhalation give us much more valuable information in regard to the protection afforded by vaccination against pneumonic plague than do those performed with the guinea pigs. In the instances where the infection was severe and all of the control unvaccinated monkeys succumbed to pneumonic-plague infection, only 9 per cent of the vaccinated monkeys survived.

[5] See IV, p. 176 of this report.

In conclusion, our experiments have demonstrated that vaccination does not afford the same protection against pneumonic plague that it does against bubonic plague in experimental animals. They indicate strongly that prophylactic inoculation can not be relied upon as even a reasonable means of protection against pneumonic infection in man. It would appear that a proper mask furnishes the only reliable method of protection.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

X. IMMUNIZATION OF GUINEA PIGS BY VACCINATION WITH AVIRULENT PLAGUE BACILLI MIXED WITH AGAR.

By M. A. BARBER.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

The object of this research was to test the behavior of doses of an avirulent organism when mixed with agar and to compare the immunizing effect of such doses with that of the organism alone. Plague was chosen as a convenient test organism since it grows well in agar and has been shown by Strong [1] and Kolle and Otto [2] to immunize about 70 to 83 per cent of guinea pigs when given alone in doses of 1 to 2 agar-slant-cultures.

The mixture of the avirulent organism with agar aims at the following possible advantages:

1. A very small initial dose may be given, lessening the dangers of anaphylactic or other intoxication; 2, the gradual absorption of agar, and with it the growing colonies of bacteria and their products insures dosage that is gradual and, for a time, gradually increasing; 3, one inoculation may insure a dosage extending over considerable time; 4, a local reaction is set up by the agar which, in some cases, might favorably affect immunization.

The technique is simple. Nutrient agar of the ordinary sort, or, in some cases, made somewhat stiffer than usual, was melted, cooled to about 43°C., and a known amount of bacilli thoroughly mixed with it. Quantities of the still liquid mixture, varying

[1] *This Journal, Sec. B* (1907), 2, 155.
[2] *Ztschr. f. Hyg. u. Infectionskrankh.* (1903), 45, 512; 48, 399.

from 0.25 to 6 cubic centimeters, were inoculated both subcutaneously and intraperitoneally by means of an ordinary syringe. Especial precautions were taken against contamination; for a contaminating organism might develop rapidly under protection of the agar. The escape of the still liquid agar could be prevented by applying ice or cold water to the point of inoculation after withdrawing the needle.

It was found that if the agar mass was deposited immediately under the skin, necrosis of the overlying skin sometimes occurred. This could be avoided by depositing the agar well into the subcutaneous tissues. Few cases of death due to a contaminating organism occurred.

In order to observe the behavior of the inoculations, animals, some of them inoculated with agar alone and some with the agar pest mixture, were sacrificed at the following periods after inoculation: three and one-half hours, twenty-one hours, forty-eight hours, three days, four days, five days, six days, seven days, twenty-one days, and twenty-nine days. In all cases the agar mass with the surrounding tissues was studied in frozen sections.

The agar mass examined soon after inoculation was found to be permeated by connective tissue fibers more or less stretched by the agar. Within a few hours leucocytes begin to invade the mass, following the larger strands of connective tissue. They become more and more abundant as time goes on, and within two or three days part of the mass may become semifluid. This was more marked in the agar and pest inoculations. Areas of agar could be detected up to the twenty-ninth day, at least. Colonies of plague, some of them 30 $\mu\mu$ in diameter, were found scattered in the agar mass as early as twenty-one hours after inoculation and as late as five days. As the time went on the agar became more and more invaded by leucocytes and the zone of granulation tissue encroached more and more on the agar, until nothing but a small hard lump of scar tissue could be felt. In some cases this lump could be felt as late as two months after inoculation. In a few cases a softer abscess persisted many weeks.

The contents of the agar mass was examined for plague bacilli in all sacrificed animals and in some living animals by withdrawing some of the mass by means of a glass capillary pipette. Living plague bacilli were detected in practically all sacrificed

animals, and in 3 cases of living animals twenty-five days after inoculation in pure culture. On account of the lack of virulence of this strain of plague, it was impossible to identify it by guinea pig inoculations; but the morphology, staining, and characteristic growth on various media, including the growth in broth covered with sterile vaseline, served to identify the organism satisfactorily. The fact that several tests showed the same plague-like organism in pure culture helped to confirm the identification. One animal inoculated with 3 cubic centimeters of agar with avirulent plague bacilli died from some unknown cause twenty-nine days after inoculation. In it the avirulent plague bacillus in apparently pure culture was found at the point of inoculation, while no growth of any sort was obtained from the spleen, liver, lungs, or peritoneal cavity. There was still a small mass of agar remaining. This case is the more remarkable since there had been some necrosis of the skin over the inoculated agar. This had entirely healed at the time of the death of the animal.

It was demonstrated then that, following a small initial dose of avirulent pest bacilli in agar, pest colonies form in the agar, a portion of agar remains as long as twenty-nine days, and that pest bacilli may be recovered in pure culture after that interval. Gradual dosage over a long period of time may, therefore, be attained by this method.

To test for immunity, the animals inoculated with the agar-avirulent-pest were subjected to infection with virulent plague bacilli. In the first group, included under Table I, relatively small amounts of pest bacilli were mixed with the agar and various quantities of agar were inoculated. The test dose in all animals included in Table I was 0.5 cubic centimeter of a suspension of a 24-hour culture made directly from an infected guinea pig. This strain had been kept at a high degree of virulence by long passage through guinea pigs and was regularly fatal to guinea pigs, inoculated cutaneously, in three to five days. The number of bacteria in the test dose was estimated by means of the Thoma Zeiss counting chamber at 750,000, counting each element of a chain as one. By plating dilutions, the test dose gave 390,000 colonies, a lower number than that obtained by counting, since a chain or united pair could give but one colony. The test dose was given subcutaneously.

TABLE I.

Number of guinea pigs inoculated.	Previous treatment.	Died of plague.	Recovered.	Average number of days of survival after inoculation in fatal cases.	Remarks.
1	Recovered from infection with virulent plague.	0	1	----------	Marked infiltration at point of inoculation.
2	Recovered from infection with plague strain "Shanghai."	0	2	----------	Marked infiltration at point of inoculation.
16	No previous immunization	16	0	4.8	
2	Avirulent pest alone (about $\frac{1}{10}$ slope).	2	0	11.0	Immunizing doses given from 30 to 60 days before test dose.
2	About $\frac{1}{10}$ loop of avirulent pest in $\frac{1}{2}$ and $\frac{1}{4}$ cc. agar.	2	0	6.5	
4	About $\frac{1}{100000}$ loop in 1 cc. ordinary agar.	3	1	5.3	
2	About $\frac{1}{100000}$ loop in 1 cc. 4 per cent agar.	1	1	3	
1	About $\frac{1}{100000}$ loop in 2 cc. 4 per cent agar.	1	0	6	All agar and pest given subcutaneously except in last-mentioned group of 5 where inoculation was intraperitoneal.
1	About $\frac{1}{100000}$ loop in 3 cc. 4 per cent agar.	1	0	3	
9	About $\frac{1}{100000}$ loop in 3 cc. 4 per cent agar.	6	3	8.8	
5	About $\frac{1}{100000}$ loop in 1 cc. ordinary agar.	2	3	5	

In the series represented by Table I, all nonimmunized controls died of plague within six days after inoculation, except one which survived nine days. The 16 in this group include 6 which were inoculated without infection some two to three months previously with single small doses of 2 to 100 plague bacilli, either virulent or of the "Shanghai" strain—a somewhat attenuated race. There was apparently no immunizing effect of these single small doses. Of the immunized group, the two which received avirulent pest bacilli alone in a considerable dose (about 1 cubic centimeter of a thin suspension) died in thirteen and nine days after the test dose. Of the 24 which had received avirulent pest in agar, 8 recovered from the test dose.

In Table II are given the results for a second group of guinea pigs. Here all animals received in immunization a much larger amount of avirulent plague in a larger amount of agar and the doses were given intraperitoneally. The test dose was in all cases given thirty-seven days after the avirulent pest agar. This test dose consisted of about 500,000 bacteria of the same highly virulent strain as that used in the first group, and was

taken from an 18-hour culture made directly from an infected guinea pig and inoculated subcutaneously.

TABLE II.

Number inoculated.	Previous treatment.	Died of plague.	Recovered.	Average number of days of survival after inoculation; fatal cases.	Remarks.
8	Highly immunized survivors of test dose, Table I.	0	8	----------	Slight local infiltration.
7	Nonimmunized ----------	7	0	5.4	
5	Avirulent pest alone. 1 slope----	1	4	11.0	
2	Avirulent pest alone. ½ slope----	2	0	4.5	
1	Avirulent pest alone. ¼ slope----	1	0	7.0	
3	Avirulent pest 1 slope in 5 to 6½ cc. ordinary agar.	2	1	6.5	
2	Avirulent pest ½ slope in 5 cc. ordinary agar.	0	2	----------	
5	Avirulent pest $\frac{2}{5}$ slope in 5 cc. ordinary agar.	3	2	6.3	

In Table II it is seen that of this group all highly immunized animals survived with but little reaction, all nonimmunized controls died, and of the 8 receiving avirulent pest alone and of the 10 receiving agar and avirulent pest one-half survived in each group. The results of this series were somewhat less favorable than that done with the smaller doses.

Summarizing the results of the two groups, it is seen that the proportion of recoveries following an immunization with this strain of avirulent plague mixed with agar (one-third in group 1 and one-half in group 2) is rather less than the proportion obtained by Strong with avirulent pest alone. In the series described here the number of controls which received avirulent pest alone was too few and the doses, for the most part, too small to give a fair comparison. These series were at first intended to serve only as preliminary ones, but the results did not seem favorable enough to warrant a further series with this strain of avirulent plague. The relative inefficacy of this method is shown especially by 3 animals inoculated subcutaneously with agar and avirulent plague. These three showed avirulent plague at the point of inoculation in pure culture twenty-five days after inoculation, yet 2 of the 3 succumbed to the test dose of three-fourths million virulent plague.

As regard the relative immunizing power of single small initial doses of avirulent plague in agar compared with much larger

doses given alone, the above series show more decisive results. While in no case was immunization to the test dose effected by less than one 24-hour agar-slant-culture of avirulent pest bacilli alone, the 22 which received only 1/10,000 to 3/10,000 of a loop mixed with agar gave 8 recoveries. This result, taken with the proof obtained of the long survival in animals of avirulent pest when inoculated with agar, gives some encouragement of success with a series inoculated with a slightly more virulent strain of plague in agar. Experiments with agar and organisms other than plague are now in progress.

Summarized briefly, the results obtained demonstrate: 1, The long persistence of ordinary nutrient agar mixed with avirulent plague bacilli and inoculated subcutaneously in guinea pigs (twenty-nine days); 2, the long survival of avirulent plague bacilli in pure culture in such inoculation (twenty-five days and twenty-nine days); 3, the possibility of immunizing a proportion of animals with very small initial doses of avirulent plague bacilli in agar (1/10,000 loop).

The results indicate that as good or better success may be obtained in immunizing with living avirulent plague bacilli alone as with doses mixed with agar. This is true of the strain of avirulent plague used. The method might give better results with another strain of avirulent plague or in immunization with some other organisms.

ADDENDUM.

It was noted that even with highly immunized animals which had survived a severe infection with virulent plague some local reaction followed the test doses of avirulent plague. This was especially true of the animals in Table I receiving a test dose of three-fourths million. It was thought worth while to ascertain if a smaller dose would give in highly immunized animals such local reaction. Ten animals of the series described in Table I, which had survived an infection following a dose of three-fourths million avirulent bacilli, were inoculated subcutaneously with doses of from 45 to 80 virulent plague bacilli. A similar dose was fatal to 7 out of 10 nonimmunized controls. None of the three noninfected controls or of the 10 immunized animals showed a local reaction greater than that which could be accounted for by the prick of the very fine capillary pipette used in inoculating.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

XI. THE INFECTION OF GUINEA PIGS, MONKEYS, AND RATS WITH DOSES OF PLAGUE BACILLI, RANGING FROM ONE BACILLUS UPWARDS.

By M. A. Barber.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

The method of isolation and inoculation of minute doses of bacilli has been described in various papers[1] by the writer and needs but a short description here.

The organisms to be manipulated are suspended in hanging drops of liquid under a large cover glass. This cover is placed over a moist chamber consisting of a glass box open at one end, and the whole is mounted on the stage of the microscope. A glass pipette, the end of which is drawn into a microscopically fine capillary point, is attached to a special holder clamped to the stage of the microscope. The capillary point, bent upward at right angles, is raised by the holder into the hanging drop containing the bacilli, both bacilli and point being kept in view in the field of the microscope. The bacilli enter the point by capillarity and may then be discharged on a sterile part of the cover, or on a new cover, by blowing through a rubber tube attached to the outer end of the pipette. Doses thus isolated are taken up by a new sterile pipette and inoculated. The point is made to pierce the skin of the animal, and the dose is injected by blowing into the rubber tube attached to the pipette. Enough salt solution is drawn into the inoculation-pipette before taking up the dose to wash out the bacilli during the discharge, and just before inoculation, enough sterile salt solution is drawn in to wash the bacteria some distance back from the tip. This is done to prevent the loss of the dose by the breaking off of

[1] *Sci. Bull.*, Kansas Univ. (1907), 4, 3. *Journ. Infect. Dis.* (1907), 5, 380; (1906), 6, 634; (1911), 8, 348.

the more delicate part of the tip during inoculation. That the bacteria pass into the animal with the inoculating fluid and do not remain behind in the pipette has been shown by a series of controls where organisms were discharged into a suitable culture-medium instead of into the animal.

Most of the inoculations were made with a virulent strain of plague which has been fatal to guinea pigs usually in three to five days after cutaneous inoculaton. In the series "Shanghai," a strain of somewhat less virulence was used.

Bacilli were taken immediately from the blood or spleen of infected animals or from the first cultures made from these sources. In most cases bacilli from the heart's blood or organs were grown for a few hours in hanging drop or test-tube in a mixture of body fluid and water of condensation of ordinary agar. When the formation of chains showed that the organisms were multiplying, they were isolated and inoculated at once.

Doses of 50 or less were carefully counted and larger doses were counted or closely estimated; but in the summary given here all doses are arranged in convenient groups. The doses of one-half and three-fourths million were estimated by the Thoma Zeiss counter. In the column giving the dosage, a pair of very short elements clinging closely together were, in some cases, reckoned as a single bacillus; though probably each element was capable of individual growth.

All animals were inoculated subcutaneously, the guinea pigs and monkeys under the skin of the abdomen, the rats at the root of the tail.

TABLE I.—*Guinea pigs and virulent plague.*

Dose in number of bacilli.	Number of animals inoculated.	Result.			
		Infected and died.	Infected and recovered.	Not infected.	Average number of days between inoculation and death.
1	9	6	0	3	9.7
2 to 10	7	3	0	4	9.0
11 to 50	14	10	0	4	8.6
51 to 100	1	1	0	0	7.0
101 to 200	3	2	1	0	9.0
201 to 500	2	2	0	0	9.0
2,000	1	1	0	0	12.0
½ million	4	4	0	0	6.5
¾ million	16	16	0	0	4.8

Total number receiving doses of 1 to 500, 36. Per cent of fatal infections, 63.9.

TABLE II.—*Guinea pigs and plague strain "Shanghai."*

Dose in number of bacilli.	Number of animals inoculated.	Result.			
		Infected and died.	Infected and recovered.	Not infected.	Number of days between inoculation and death.
1	1	0	0	1	
2 to 10	6	1	0	5	14
11 to 50	1	0	0	1	
51 to 100	1	1	0	0	12
101 to 200	1	0	1	0	
201 to 500	3	1	2	0	8
Total	13	3	3	7	

TABLE III.—*Monkeys and virulent plague.*

Dose in number of bacilli.	Number of animals inoculated.	Results.			
		Infected and died.	Infected and recovered.	Not infected.	Number of days between inoculation and death.
1	12	2	0	10	8 and 15
2 to 10	10	1	0	9	17
11 to 50	4	1	0	3	5
101 to 200	2	2	0	0	20 and 9
201 to 500	3	0	0	3	
Total	31	6	0	25	

TABLE IV.—*Rats and virulent plague.*

Dose in number of bacilli.	Number of animals inoculated.	Results.			
		Infected and died.	Infected and recovered.	Not infected.	Number of days between inoculation and death.
1	2	0	0	2	
2 to 10	7	2	0	5	9 and 13
11 to 50	4	2	0	2	9 and 19
51 to 100	1	0	0	1	
Total	14	4	0	10	

It will be noticed from Table I that the percentage of fatal infections in the guinea pigs receiving a dose of one bacillus of the virulent strain is nearly the same as that of the entire 36 which received 1 to 500; namely, 63.9 per cent and 66.7 per cent respectively.

The "Shanghai" strain, though evidently less infective, was in one case fatal in a dose of 5 bacilli. These results illustrate how large a part varying susceptibility of animals plays in infection, and emphasize the necessity of inoculating a series of animals in any test of virulence of a microörganism.

In some guinea pig inoculations, one or few bacilli were washed with salt solution before inoculating and some of these washed doses resulted in fatal infection. Since a most minute quantity, if any at all, of the original body fluid was then inoculated, it does not seem probable that aggressins played any part in these infections. In the experiments on monkeys few succumbed to the small doses, though in two cases fatal infection followed a dose of one bacillus.

In the rat series the wild gray rat was used. One animal succumbed to a fatal infection following a dose consisting of one chain of four small elements, another to a dose of three bacilli, each divided into two still adherent elements. Two other rats succumbed to doses of 50 and 60, respectively, of such pairs. These results render more plausible the view that sufficient bacilli for infection may enter the abraded skin from the fæces of a flea or a crushed flea. The English Plague Commission has shown that flea fæces or flea bodies may contain very large numbers of plague bacilli.

On the average, animals infected with minute doses survived nearly twice as long as those infected with three-fourths of a million or more.

In summary, these results show conclusively that the smallest possible dose of virulent plague bacilli may infect fatally the more susceptible guinea pigs, monkeys, or rats.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

XII. SOME EXPERIMENTS TO DETERMINE THE EFFICACY OF VARIOUS MASKS FOR PROTECTION AGAINST PNEUMONIC PLAGUE.

By M. A. Barber and Oscar Teague.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

During the epidemic of pneumonic plague which raged in Manchuria during the winter of 1910 to 1911, it was believed and, toward the close of the epidemic, was experimentally demonstrated, by Strong and Teague, that sputum in the form of invisible droplets containing viable plague bacilli was frequently suspended in the air near the coughing pneumonic-plague patients. A Petri dish, containing solidified agar-culture-medium, held for a minute or two before the mouth of a patient and closed after a single cough did in some instances on incubation show numerous colonies of plague bacilli, although no visible particles of sputum had been thrown against it.[1] There was every reason to believe that even the smallest number of these bacilli inhaled into the lung would lead to infection and that this was, in fact, the common mode of infection in pneumonic plague. The obvious method to protect against such infection was to interpose a barrier to the passage of these droplets into the mouth and nostrils. With this object in view, masks were worn quite generally by physicians and attendants when in the presence of plague patients or suspected cases. That protection was afforded by the masks apparently went unquestioned and, without the sense of security that their use gave, the mental strain in connection with the work would have been almost unbearable.

The total number of deaths that occurred among physicians,

[1] Report of the International Plague Conference held at Mukden, April, 1911. Manila (1912), 83. See also II, p. 137 of this report.

nurses, attendants, and inspectors during the recent epidemic of pneumonic plague in Manchuria will never be known. The following death roll at Fuchiatien, the Chinese city near Harbin, shows that the total must have been extremely high.

List of deaths of antiplague staff at Fuchiatien.[2]

Doctors	1 out of 20
Students	1 out of 29
Native practitioners	4 out of 9
Police inspectors	2 out of 31
Police	30 out of 688
Sanitary police	11 out of 206
Mounted police	5 out of 80
Firemen	5 out of 20
Coolies	102 out of 550
Cooks	4 out of 60
Ambulance parties	69 out of 150
Soldiers	63 out of 1,100
Total	297 out of 2,943

In South Manchuria the plague sanitary corps suffered a loss of 122 persons among whom were 1 Japanese, 1 English, and 40 Chinese physicians. This represents 2.66 per cent of the total plague mortality in the districts concerned.[3]

The presumption is that all of the members of the sanitary corps wore masks. The masks were, however, not worn constantly nor were they always properly adjusted; coolies were often seen with the masks hanging around their necks instead of being over their mouths. Hence the high death rate of the sanitary staff can not be regarded as proof of the inefficiency of masks.

In Mukden the mask which was almost universally employed consisted of a pad of absorbent cotton about 16 by 12 centimeters and about 1.5 centimeters thick; this was wrapped in gauze, the ends of which were tied at the back of the head. (See Plate V, fig. I. B.) A many-tailed bandage (see Plate V, fig. I. A) composed of three layers of gauze with holes for the eyes was tied around the entire head and served to press the mask firmly against the face and to keep it snugly in place for hours at a time. When first put on, this mask was decidedly uncomfortable, but after a few minutes one became somewhat accustomed to it and could wear it for two or three hours at a time. There was, however, always an intense feeling of

[2] *Ibid.*, p. 242.

[3] *Ibid.*, p. 244.

relief on removing it. We shall designate this type of mask in the discussion to follow as the "Mukden mask." [4]

The following experiments were undertaken with the idea of determining whether this Mukden mask is, in fact, an efficient barrier against the passage of plague bacilli into the lungs and, also, whether or not other types of masks are more efficient.

At the International Plague Conference held in Mukden in April, 1911, Broquet, the French delegate, demonstrated a mask "copied from those used by doctors in the epidemic of the fourteenth century as shown in old books." [5] It consisted of a hood of light canvas or khaki cloth, covering the entire head and drawn in at the neck. In front was a window of mica. No experiments had been performed to test the efficacy of this mask. We shall refer to this type of mask hereafter as the "Broquet mask." It was not used during the recent epidemic of pneumonic plague in Manchuria with the exception of a few times by Broquet himself.

Our preliminary tests indicated that a hood of heavy Canton flannel with a nap was more effective in holding back *Bacillus prodigiosus* than hoods of lighter cloth such as the one demonstrated by Broquet. Instead of mica for the window, we used sheet celloidon such as one sees in the storm curtains of automobiles. The hood was made narrow at the neck so that it would spread out over the shoulders and could be drawn in and tied snugly around the neck. Comparative experiments were made with this mask and the Mukden mask; the subjects wearing the two masks were forced to breathe air containing *Bacillus prodigiosus* simultaneously for the same length of time.

Bacillus prodigiosus was selected for the experiments as being entirely harmless and easily recognizable on account of its pigment production. An ordinary throat atomizer was used for making the spray, but with the idea of getting smaller droplets the rubber bulb was removed and a stronger airblast was obtained by using an automobile pump.

Special precautions were taken to avoid accidental contamination with *B. prodigiosus* on removing the mask. (See Plate V, fig. 2.) The subject was clothed in an operating gown and, in the case of the Mukden mask, his head was covered with a cloth and the eyes protected by automobile goggles. The spraying was generally done in a small, single-roomed stable which

[4] We were informed that this mask was extensively used in Harbin before its introduction into Mukden.

[5] Report of the International Plague Conference, p. 303.

was boarded up on all sides to keep out the light and to avoid, to a certain extent, currents of air. The gowns, goggles, and head-cloths were removed after the subjects had left the stable and before they entered the laboratory building. One of the authors attended to the spraying and exposure of the subjects, the other endeavored to keep himself and his laboratory room free from *B. prodigiosus* and made the necessary plate-cultures in order to determine the result of the test. At first the saliva, taken before and after the spraying, was smeared over agar plates, but later it was found that small pieces of moistened cotton, placed in the nostrils and before the mouth (underneath the Mukden mask), rendered the test much more delicate.

Agar plates were exposed during the course of the experiment in order to obtain an indication of the *living* prodigiosus bacilli that were in the air around the mask at that time.

The following protocols, selected from a long series of such experiments, demonstrate the general mode of procedure and the results obtained.

PROTOCOL NO. 1. (EXPERIMENT NOS. 97 AND 98.)

Two laboratory boys[6] served as subjects. Control plates were made as follows: A quantity of saliva was expectorated into a plate containing solidified agar, distributed by means of a sterile cotton plug, and a small

[6] The first experiment was performed upon ourselves to demonstrate the harmlessness of the procedure. Then several of our colleagues and about 8 different laboratory boys served as subjects in these experiments. Yet, owing to the large number of experiments that were done, it was found necessary to use the same laboratory boys repeatedly as subjects. However, a period of at least a week was allowed to elapse before a boy was again called upon to serve, and then smears were made from nostrils and saliva to determine whether by any chance *Bacillus prodigiosus* was present. These tests proved to be in every case negative. In order to gain some idea of the length of time that *Bacillus prodigiosus* can persist in the mouth, one of us rinsed his mouth with a suspension of prodigiosus (one slant in 10 cubic centimeters of salt solution) and gargled some of the same suspension. Plates inoculated with his saliva at intervals gave the following results:

Saliva after three-fourths hour	Plate No. 1: Overgrown with prodigiosus.
Saliva after 3½ hours	Plate No. 1: Overgrown with prodigiosus. Plate No. 1: Overgrown with prodigiosus.
Saliva after 5½ hours	Plate No. 1: Overgrown with prodigiosus.
Saliva after 16 hours	Plate No. 1: 20 colonies of prodigiosus. Plate No. 2: 15 colonies of prodigiosus.
Saliva after 19½ hours	Plate No. 1: No colonies of prodigiosus. Plate No. 2: No colonies of prodigiosus.

Two meals were taken during the course of this experiment.

portion spread thinly over a second agar plate. Both plates were preserved for growth and examination. At the same time the nostrils were swabbed with a small pledget of sterile cotton moistened with salt solution and this rubbed over solidified agar. (In no case was *B. prodigiosus* obtained from the nostrils or saliva in these controls taken before spraying.)

The boys were clothed with operating gowns.

Boy No. 1 wore a Mukden mask consisting of two and one-half layers of Johnson and Johnson absorbent cotton. Thin layers of this cotton in Petri dishes were steamed in an Arnold sterilizer and then placed in the ice box so that water would condense upon the cotton and inside of the Petri dishes. A portion of the cotton thus moistened was placed in approximately the center of the mask between the layers of the cotton, so that when the mask was in place it lay before the mouth and nostrils. Small bits of the moist cotton were placed within the nostrils and a larger piece before the mouth and nostrils. This latter piece was held in place by the mask. Small pieces of dry absorbent cotton were placed on each side of the nose and then the Mukden mask was tied in place. Automobile goggles were worn over the eyes. The exposed portion of the head above the mask was covered with a cloth.

Boy No. 2 wore a Broquet mask of heavy Canton flannel cloth. This hood had been used in a number of previous experiments after each of which it had been disinfected in lysol solution and placed in the sun to dry. Small bits of the steamed moist cotton were placed loosely within the nostrils, and as in the preceding instance a larger piece of the same cotton was placed over the mouth and nostrils. This was held in place by a strip of gauze which was tied at the back of the head. A straw hat was placed on the boy's head, and the mask was then put on and tied in snugly around the neck. (See Plate V, fig. 2.)

The two boys, thus masked, were taken into a stable with the walls boarded up to keep out the light and to prevent currents of air. A suspension of prodigiosus bacilli in 0.5 per cent sodium chloride solution (1 agar slant in about 40 cubic centimeters) was sprayed by means of a throat atomizer connected with an automobile pump. The spray was directed alternately toward one mask and then the other for a period of three minutes. The boys were then brought back to the laboratory. But the gowns, goggles, and head-cloths were removed before they entered the laboratory building which was only a few meters away. This was done in order to prevent a possible contamination of the test culture plates with prodigiosus bacilli which might have become scattered in the air while the masks were being removed. (The gowns and cloths were sterilized in an autoclave at 120°C. before they were used in the next experiment.)

The subjects then proceeded to the door of the laboratory room where the masks were removed and cultures made as follows:

The cotton taken from before the mouth, that from the nostrils, and, in case of the Mukden mask, that from the interior of the mask were transferred by sterile forceps to separate Petri dishes containing solidified agar, and rubbed over the surface of the agar. Each mass of cotton was then transferred to a second Petri dish well wet with salt solution and rubbed over the second plate and left on the surface of the media.

The cotton was wet in order to afford conditions for growth to any *B. prodigiosus* which might otherwise have remained in the dry center of the cotton. (In a few cases the wet cotton mass alone, after twenty-four hours, showed the red color indicative of the growth of *B. prodigiosus*.) All plates were left in the dark at room temperature (25° to 30°C.), protected by glass jars.

The result of all the cultures, read two days later, was as follows:

Boy No. 1. Mukden mask.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus *present.* Plate No. 2: Prodigiosus *present.*
Cotton before mouth after exposure	Plate No. 1: Prodigiosus *present.* Plate No. 2: Prodigiosus *present.*
Cotton within the mask after exposure	Plate No. 1: Prodigiosus *present.* Plate No. 2: Prodigiosus *present.*

Boy No. 2. Canton flannel Broquet mask.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils before exposure	Plate No. 1: Prodigiosus absent. Plate No. 1: Prodigiosus absent.
Cotton before mouth after exposure	Plate No. 1: Prodigiosus *present.* Plate No. 2: Prodigiosus *present.*
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus *present.* Plate No. 2: Prodigiosus *present.*

A plate exposed to the air of the laboratory room, while the above plates were being prepared, showed no red colonies.

DISCUSSION OF PROTOCOL NO. 1.

This experiment shows that neither the Mukden mask nor the heavy Canton flannel Broquet mask is able to hold back completely prodigiosus bacilli when they are sprayed in large numbers continuously for a period of three minutes about the heads of the subjects. As this Broquet mask is the most efficient of all the masks with which we have experimented, it follows that none of our masks can withstand this test. The fact that the moist cotton from the center of the Mukden mask contained many prodigiosus bacilli shows that some of the prodigiosus bacilli passed directly through the mask; or, in other words, that the inefficiency of this mask is not due solely to the fact that the bacilli pass around the edges of the cotton pad or through the free spaces at the sides of the nose which were,

perhaps, only imperfectly plugged with cotton.[7] In this experiment the masks are subjected to a much more severe test than would occur in practice; nevertheless, it presents conclusive evidence, we believe, that these masks do not offer absolute protection against infection with pneumonic plague.

PROTOCOL NO. 2. (EXPERIMENTS NOS. 69 AND 70.)

February 3. A fresh culture of *B. prodigiosus* upon slanted agar was suspended in 0.5 per cent sodium chloride solution and about one-half of this suspension was sprayed through an atomizer by means of an automobile pump. The spray was directed toward all parts of a small single-roomed stable with the walls boarded up to keep out the light and, to a certain extent, the currents of air. Three minutes after the spraying had been discontinued, two subjects, one wearing our Canton flannel Broquet mask and the other a "Mukden mask" were taken into the room and allowed to remain for ten minutes. The temperature of the stable measured 28° 5 C. The weather was overcast and there had been a drizzling rain of short duration about one hour before the experiment began.

Number of living B. prodigiosus in the air.

	Time after spraying.	Number of prodigiosus colonies.
Subjects exposed.	½ to 3 minutes	Innumerable.
	3 to 6½ minutes	11,400
	6½ to 9 minutes	1,416
	9 to 12 minutes	472
	12 to 23 minutes	63

Subject No. 1. Canton flannel Broquet mask.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent.
	Plate No. 1: Prodigiosus absent.
Cotton from nostrils before exposure	Plate No. 1: Prodigiosus absent.
	Plate No. 2: Prodigiosus absent.
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus absent.
	Plate No. 2: Prodigiosus absent.
Cotton before mouth after exposure	Plate No. 1: Prodigiosus absent.
	Plate No. 2: Prodigiosus absent.

[7] In another experiment we obtained further evidence that bacteria may pass directly through the cotton pad of the Mukden mask. Layers of cotton as thick as the Mukden mask, sufficiently wide to cover the entire face and overlapping at the back of the head, were held in place by a many-tailed bandage, no openings in the cotton or bandage being made for the eyes. The remaining portion of the subject's head and his neck were then bandaged with layers of cotton of the same thickness and a suspension of prodigiosus bacilli was sprayed about his head for a period of seven minutes. The bacilli were recovered from the cotton immediately before his mouth and from his saliva.

Subject No. 2. Mukden mask.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus *present.* Plate No. 2: Prodigiosus *present.*
Cotton before mouth after exposure	Plate No. 1: Prodigiosus *present.* Plate No. 1: Prodigiosus *present.*
Cotton within the mask after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.

DISCUSSION OF PROTOCOL NO. 2.

Living prodigiosus bacilli were very numerous at the beginning of the test, but decreased very rapidly during the ten minutes that the subjects were exposed. This must be regarded also as a very severe test, though by no means so severe as the preceding one. The Broquet mask withstood the tests, while the Mukden mask failed to hold back all the prodigiosus bacilli. This experiment, therefore, demonstrates clearly the superiority of the Broquet mask over the Mukden mask.

PROTOCOL NO. 3. (EXPERIMENTS NOS. 67 AND 68.)

February 1, 1912. Spraying as the preceding experiment. The two masked subjects were taken into the stable six minutes after the spraying had been discontinued and allowed to remain ten minutes. Hot, sunshiny day. Temperature in the stable 29°C.

Number of living prodigiosus bacilli in the air.

	Time after spraying.	Number of prodigiosus colonies.
Subjects exposed.	1 to 3 minutes	6,000
	3 to 6 minutes	2,760
	6 to 9 minutes	280
	9 to 12 minutes	127
	12 to 15 minutes	29
	15 to 30 minutes	1

Subject No. 1. Mukden mask.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton before mouth after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus *present.* (Cotton red and 32 colonies.)
Cotton within the mask after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.

Subject No. 2. Canton flannel Broquet mask.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton before mouth after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.

DISCUSSION OF PROTOCOL NO. 3.

This test was an extremely light one. A Petri dish exposed during the first three minutes that the masked subjects were in the room developed only 280 prodigiosus colonies and another, during the last three minutes, only 29 colonies. In spite of the small number of living prodigiosus bacilli that were in the air, the Mukden mask failed to hold back all of them. We are inclined to believe that this test is even a less severe one than that to which the masks were subjected during the recent plague epidemic in Manchuria, as the coughing patients in the crowded wards must have been throwing out hundreds of fine droplets almost continuously and, on account of the low temperature, the plague bacilli in these droplets must have remained suspended in the air in a viable condition for a considerable period of time. Since we have found repeatedly in tests which were not severe that the Mukden mask allowed bacilli to pass, we are forced to the conclusion that the sense of security felt by those who wore this mask in the Manchurian epidemic was not justified.

PROTOCOL NO. 4.

This experiment was carried out in a cold-storage room measuring about 2.5 by 3 meters at a temperature of 12°C. A 24-hour agar-culture of prodigiosus was suspended in about 40 cubic centimeters of 0.5 per cent sodium chloride solution and filtered twice through cotton. A portion of this suspension was sprayed by means of a throat atomizer connected by rubber tubing with a two-cylinder force-pump such as is used in filling automobile tires. The spraying was continued for a period of two minutes, the spray being directed toward all portions of the room. The pump was then removed and the door of the cold room quickly closed. A period of two hours was allowed to elapse, and then the three masked boys were hurried into the room and the door was closed behind them. They remained ten minutes in the room. During this time each held in his hand an open Petri dish containing solidified agar and closed it immediately after leaving the cold room.

Boy No. 1 wore a Mukden mask, boy No. 2 our Canton flannel Broquet mask. The usual measures against accidental contamination with *B. prodigiosus* were adopted. Boy No. 3 wore a mask of wet gauze. Strips of

gauze were boiled and while still warm were squeezed out and applied loosely over the lower portion of the face from the eyes to below the chin. The gauze was not in layers but was placed irregularly as in surgical dressings which are intended to absorb pus. A many-tailed bandage with holes for the eyes, such as is used with the Mukden mask, pressed the moist gauze firmly against the face and held it snugly in place. This mask was about five or six centimeters thick over the mouth and became thinner toward the edges. Goggles were worn by this boy also and the top of his head was covered with a cloth reaching down to the mask.

Boy No. 1. Mukden mask.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils before exposure	Prodigiosus absent.
Cotton within mask after exposure	Plate No. 1: Prodigiosus present. Plate No. 2: Prodigiosus present.
Cotton before mouth after exposure	Plate No. 1: Prodigiosus present. Plate No. 2: Prodigiosus present.
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus present. Plate No. 2: Prodigiosus present.
Plate held by boy during exposure	4,420 prodigiosus colonies.

Boy No. 2. Broquet mask.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils before exposure	Prodigiosus absent.
Cotton before mouth after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Plate held by boy during exposure	4,000 prodigiosus colonies.

Boy No. 3. Mask of wet gauze.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils before exposure	Prodigiosus absent.
Cotton before mouth after exposure	Plate No. 1: Prodigiosus present. Plate No. 2: Prodigiosus present.
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus present. Plate No. 2: Prodigiosus present.
Plate held by boy during exposure	4,485 prodigiosus colonies.

DISCUSSION OF PROTOCOL NO. 4.

In spite of the long interval (two hours) which elapsed between the spraying and the exposure of the subjects, this test must be regarded as a very severe one, for the plates show that numerous living prodigiosus bacilli still remained suspended in

the air at the time of the exposure. Furthermore, the number of living bacilli in the air in the cold room remains practically constant during the ten minutes of the test, while, as we have seen, in the warm stable there is a rapid decrease. This experiment shows again the superiority of our Broquet mask over the Mukden mask. It also proves that prodigiosus bacilli may pass directly through the cotton pad of the Mukden mask, for a piece of moist cotton placed near the center of the pad contained prodigiosus bacilli after the test. The mask of wet gauze also failed to hold back all the bacilli and is hence inferior to our Broquet mask. The experiment does not afford any evidence as to the relative efficiency of the Mukden mask and the mask of moist gauze.

PROTOCOL NO. 5.

March 1, 1912. The mouth of one of us was rinsed with sterile salt solution and then about 10 cubic centimeters of saliva were collected in a sterile test tube. One slant of a fresh prodigiosus culture was suspended in this saliva. The resulting suspension was thoroughly shaken and then taken a little at a time into the mouth and made into a spray by being blown between the lips. The spraying was done in a cold storage room at 9° C. The room was then kept closed for one hour, when the three masked subjects were quickly taken in and the door closed behind them. They remained inside ten minutes, each subject holding during that time an open Petri dish of solidified agar.

The masks were removed and cultures made as in the preceding experiment.

Number of living prodigiosus bacilli in the air.

	Number of prodigiosus colonies.
Plate held by subject No. 1	2,340
Plate held by subject No. 2	2,405
Plate held by subject No. 3	3,120

Subject No. 1. Mukden mask.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils before exposure	Plate No. 1: Prodigiosus absent.
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus *present.* Plate No. 2: Prodigiosus *present.*
Cotton before mouth after exposure	Plate No. 1: Prodigiosus *present.* Plate No. 2: Prodigiosus *present.*
Cotton within the mask after exposure	Plate No. 1: Prodigiosus *present.* Plate No. 2: Prodigiosus *present.*
Saliva taken after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.

Subject No. 2. Canton flannel Broquet mask.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils before exposure	Plate No. 1: Prodigiosus absent.
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton before mouth after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Saliva taken after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.

Subject No. 3. Mask of wet gauze.

Saliva taken before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils before exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton from nostrils after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.
Cotton before mouth after exposure	Plate No. 1: Prodigiosus *present*. Plate No. 2: Prodigiosus *present*.
Saliva taken after exposure	Plate No. 1: Prodigiosus absent. Plate No. 2: Prodigiosus absent.

DISCUSSION OF PROTOCOL NO. 5.

This experiment was designed to approximate more nearly to the conditions that occurred in Manchuria. It seemed possible that the viscid sputum of pneumonic plague might form larger droplets than the salt solution of our experiments and on that account be unable to pass through the masks. Preliminary tests were made by taking prodigiosus bacilli into the mouth and then holding Petri dishes containing solidified agar immediately before the mouth while talking or coughing. It was found that under these conditions prodigiosus bacilli were emitted in too small numbers and too inconstantly for the method to be satisfactory in testing our masks. Swabbing the vocal cords with the bacilli might have given satisfactory results, but this was not tried. Instead of this, it was decided to blow saliva containing prodigiosus bacilli between the lips thus converting it into a spray. The droplets of saliva produced in this way apparently passed through the masks as readily as the salt solution droplets from the atomizer. This experiment furnishes strong evidence that droplets of sputum from pneumonic-plague patients may be able to pass through the Mukden mask.

General discussion.—The protocols which have been cited could be supplemented by numerous others[8] giving similar results.

While these experiments furnish evidence that fine droplets of sputum of patients suffering from pneumonic plague may pass through the mask that was so widely used in Manchuria, yet they do not at all indicate that this mask was entirely without value. Obviously, the mask would hold back gross visible particles of sputum which are sometimes thrown out in coughing. Moreover in our experiments, when prodigiosus bacilli were recovered from the nostrils, it is probable that in the same test without the mask far greater numbers would have entered; in other words, it seems probable that great numbers of bacteria, that otherwise would have entered the nose and mouth, remain on the surface of the mask and in its substance.

Hence we believe that masks should be worn by those attending pneumonic-plague patients, but that they should not be regarded as affording absolute protection against infection; bearing this in mind, even when masked, one should remain in the near vicinity of the patient only so long as is necessary for the work in question.

CONCLUSIONS.

(1) The "Mukden mask" in general use during the epidemic of pneumonic plague in Manchuria, during the winter of 1910 to 1911, does not prevent the passage into the mouth and nostrils of *B. prodigiosus* when contained in small droplets sprayed

[8] The Mukden mask was used in 42 tests and was found to hold back the prodigiosus bacilli in only 6 of these and to allow them to pass in 36 instances. Of the 6 tests in which the bacilli failed to penetrate the mask, three were preliminary experiments to determine whether a satisfactory spray was produced in talking or coughing after rinsing the mouth with a suspension of prodigiosus bacilli; plates exposed during the experiment showed less than 20 colonies each and the method was therefore abandoned. In two others of these 6 tests the exposed plates showed only 15 and 200 colonies respectively. Finally, in the last of these 6 tests, the subject drew the cotton from before his mouth and nose into his mouth where it became saturated with saliva and plates were not made from the cotton within the nostrils.

In some of the tests in which the prodigiosus bacilli passed through the Mukden mask, the exposed plates contained only a few colonies, indicating that the test was much less severe than those in the protocols recorded above.

Our Canton flannel Broquet mask was employed in 17 different experiments. It held back all the prodigiosus bacilli in 10 of these and allowed some of them to pass in 7.

around the mask. This mask consists of a pad of absorbent cotton held over the mouth and nose by a many-tailed gauze bandage.

(2) A hood of heavy Canton flannel cloth, covering the entire head and tied in snugly at the neck, withstands much severer tests than does the Mukden mask. It does not, however, offer an absolute barrier to the passage of prodigiosus bacilli into the mouth and nostrils of the subject. This mask, with a window in front, is not more inconvenient nor more uncomfortable than the Mukden mask.

(3) It is shown that the inefficiency of the Mukden mask is not due solely to the fact that the mask fails to conform to the configuration of the face but that the bacteria may pass directly through the mask; for a piece of moist cotton placed in the center of the mask was found after the test to contain prodigiosus bacilli.

(4) It is believed that, although masks hold back many bacteria that would otherwise pass into the mouth and nostrils, nevertheless their use during the recent epidemic of pneumonic plague in Manchuria lent a *false* sense of security which may have led to the taking of unnecessary risks. We believe that these experiments fully justify the conclusion that masks such as were used in that epidemic do not offer an absolute protection against pneumonic plague.

STUDIES ON PNEUMONIC PLAGUE AND PLAGUE IMMUNIZATION.

ILLUSTRATIONS.

PLATE I.

Pneumonic plague hospital, Mukden.
FIG. 1. Side view.
2. Rear view.

PLATE II.

FIG. 1. American laboratory within courtyard of plague hospital, Mukden.
2. Room in which necropsies were performed in Mukden.

PLATE III.

FIG. 1. Ward with attendants in plague hospital, Mukden.
2. Some of the nurses and attendants outside of plague ward.

PLATE IV.

Uniform adopted for protection from infection when in contact with pneumonic-plague cases.

PLATE V.

Masks for protection against pneumonic plague.
FIG. 1. The Mukden mask.
A. The many-tailed bandage to hold the pad securely in place.
B. The cotton pad wrapped in gauze.
2. Two subjects wearing the modified Broquet mask and the Mukden mask, respectively, ready for exposure to the sprayed bacilli.

PLATE VI.

The tarbagan (*Arctomys bobac* Schreb.). (Photograph by Dr. Wu.)

PLATE VII.

FIG. 1. Lung of guinea pig which died of advanced plague infection after being exposed to air in which plague bacilli were suspended by means of spraying.
2. Lung of monkey which died of pneumonic-plague infection from inhalation; lobular pneumonia.
3. Lung of monkey which died of pneumonic-plague infection from inhalation, showing progression of lesions; lobular and lobar pneumonia.

PLATE VIII.

Human lung in pneumonic plague; marked lobar pneumonia, showing deep hyperæmia of bronchi.

PLATE IX.

Human lung in pneumonic plague, showing more well-marked areas of lobular pneumonia and pleural exudate.

PLATE X.

Human lung, pneumonic plague, showing gray hepatization and fibrinous pleurisy.

PLATE XI.

Human throat, larynx, and trachea in pneumonic plague. Marked hyperæmia of the larynx and trachea; the tonsils not swollen; marked hyperplasia of an incised lymphatic gland to the right of the trachea and of a small more hæmorrhagic gland at the base of the trachea.

PLATE XII.

FIG. 1. Lung of dog with pneumonic plague.
2. Liver of tarbagan (*Arctomys bobac* Schreb.), showing chronic plague infection.

PLATE XIII.

Microscopical section of human lung, showing particularly congestion of the alveoli filled with plague bacilli. (Drawn from magnification of 150 diameters.)

PLATE XIV.

Microscopical section of human lung, showing particularly alveoli filled with plague bacilli. (Drawn from magnification of 780 diameters.)

PLATE XV.

Microscopical section of human lung in advanced stage of pneumonia, showing bacilli in great masses about blood vessels. (Drawn from magnification of 780 diameters.)

PLATE XVI.

Microscopical section of human lung in stage of hepatization with absence of fibrin. Section stained for fibrin by Weigert's method. (Drawn from magnification of 330 diameters.)

PLATE XVII.

Microscopical section of human lung in pneumonic plague, showing character of alveolar exudate. (Drawn from magnification of 330 diameters.)

PLATE XVIII.

Microscopical section of human tonsil, pneumonic plague, showing congestion. (Drawn from magnification of 150 diameters.)

Fig. 1. Side view.

Fig. 2. Rear view.

PLATE I. PNEUMONIC-PLAGUE HOSPITAL, MUKDEN.

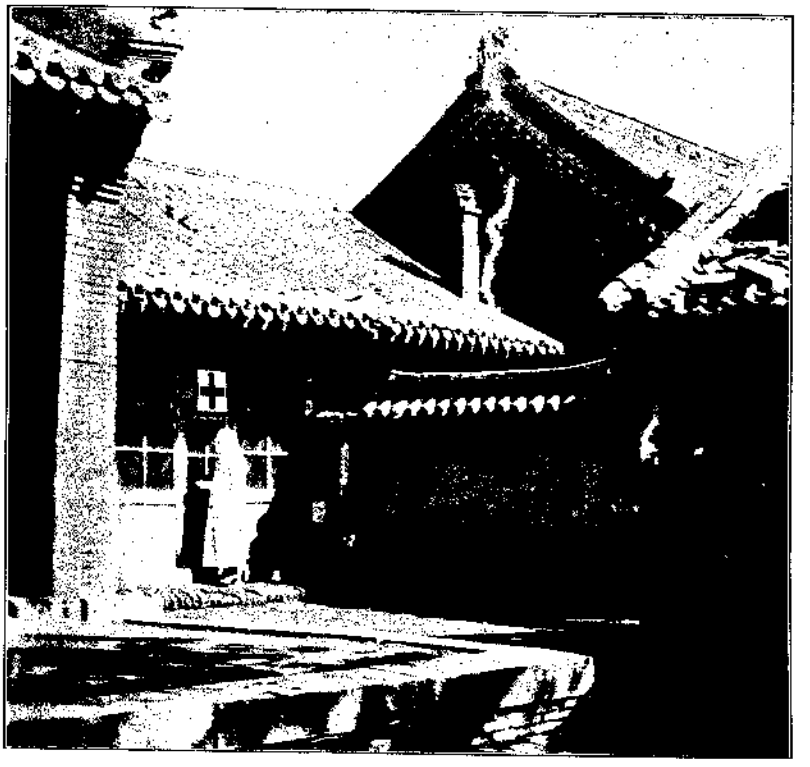


Fig. 1. American laboratory within courtyard of plague hospital, Mukden.

Fig. 2. Room in which necropsies were performed in Mukden.

PLATE II.

Fig. 1. Ward with attendants in plague hospital, Mukden.

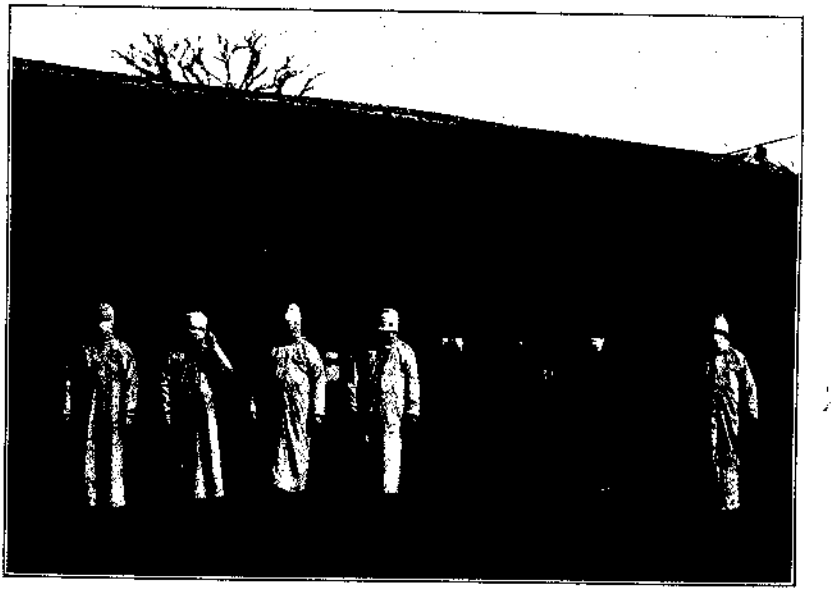


Fig. 2. Some of the nurses and attendants outside of plague ward.

PLATE III.

PLATE IV. UNIFORM ADOPTED FOR PROTECTION FROM INFECTION WHEN IN CONTACT WITH PNEUMONIC-PLAGUE CASES.

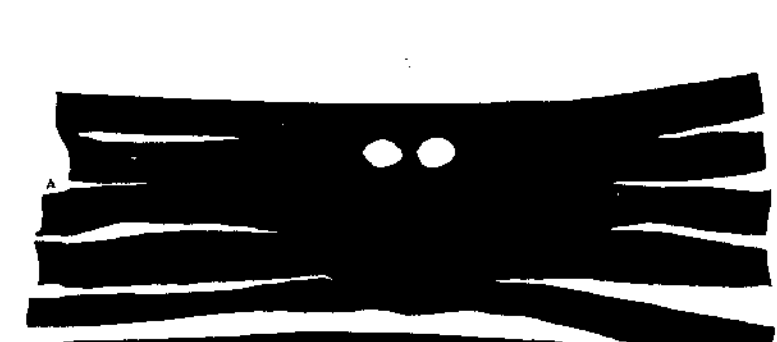
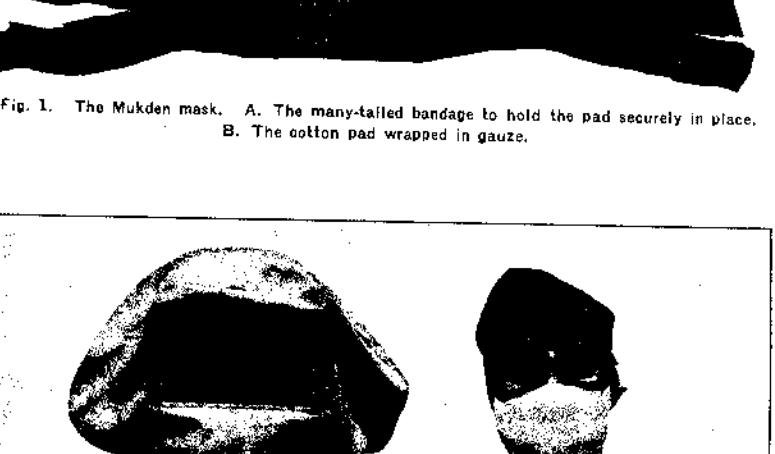


Fig. 1. The Mukden mask. A. The many-tailed bandage to hold the pad securely in place. B. The cotton pad wrapped in gauze.



g. 2. Two subjects wearing the modified Broquet mask and the Mukden mask, respectively, ready for exposure to the sprayed bacilli.

PLATE V. MASKS FOR PROTECTION AGAINST PNEUMONIC PLAGUE.

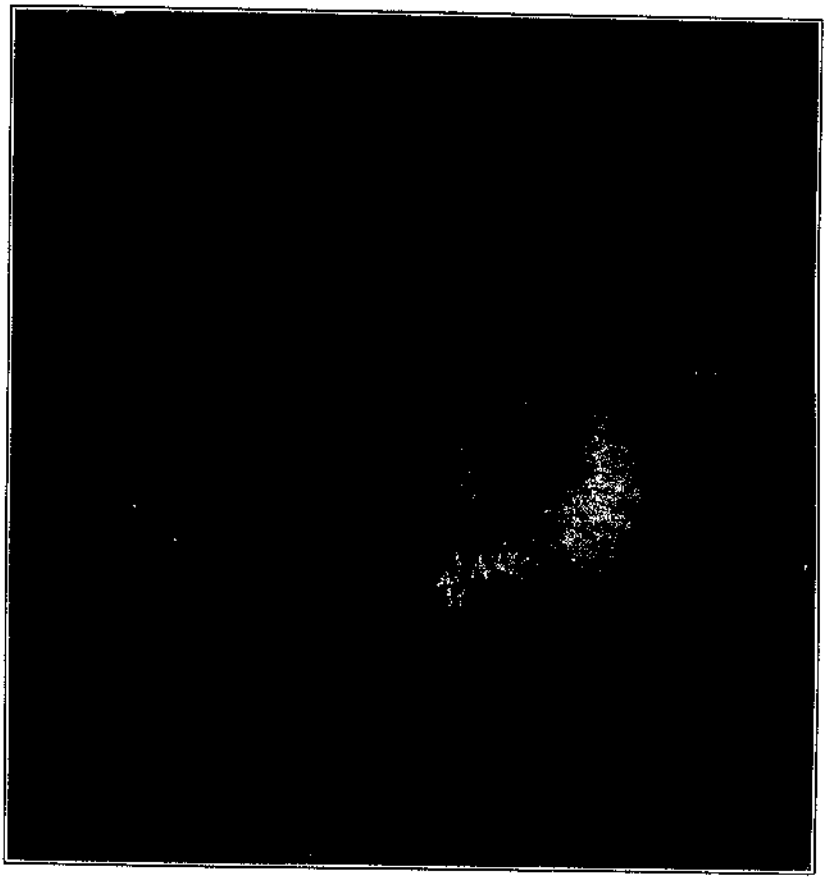


PLATE VI. The TARBAGAN (*Arctomys bobac* Schreb.).

Fig. 1. Lung of guinea pig which died of advanced plague infection, after being exposed to air in which plague bacilli were suspended by means of spraying.

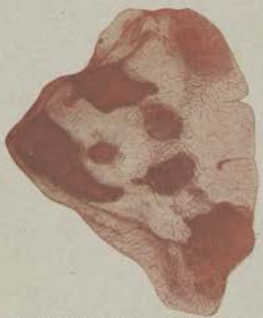


Fig. 2. Lung of monkey which died of pneumonic-plague infection from inhalation; lobular pneumonia.

Fig. 3. Lung of monkey which died of pneumonic-plague infection from inhalation, showing progression of lesions; lobular and lobar pneumonia.

PLATE VII.

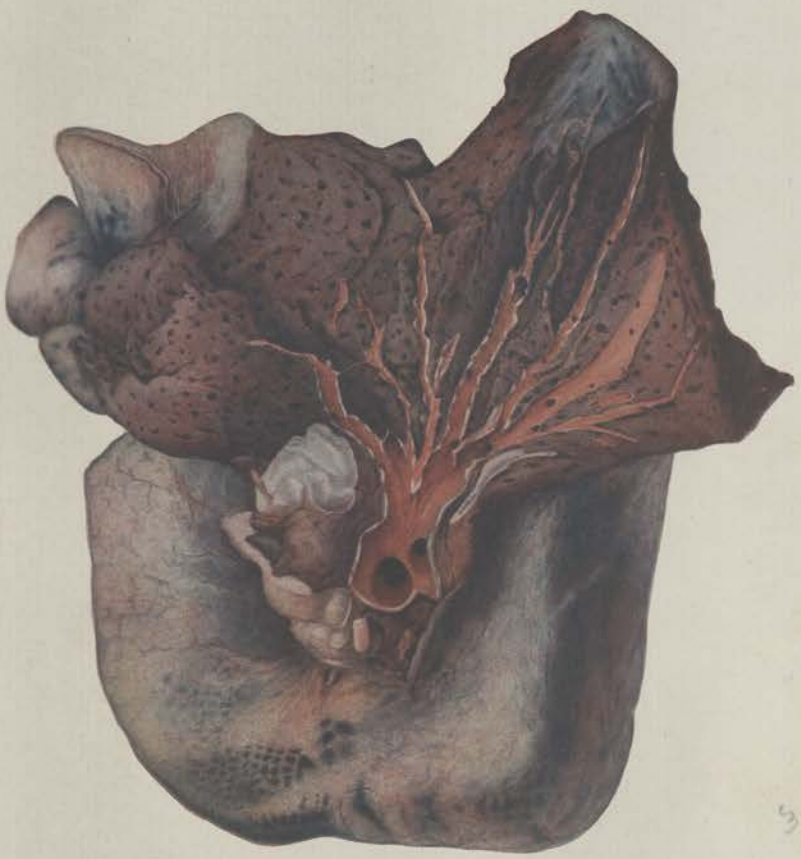


PLATE VIII.

PLATE I. LUNG IN PNEUMONIC PLAGUE. LOBAR PNEUMONIA; SHOWING DEEP HYPERÆMIA OF BRONCHI.

Plate I of the Report of the International Plague conference is reproduced here as Plate VIII.

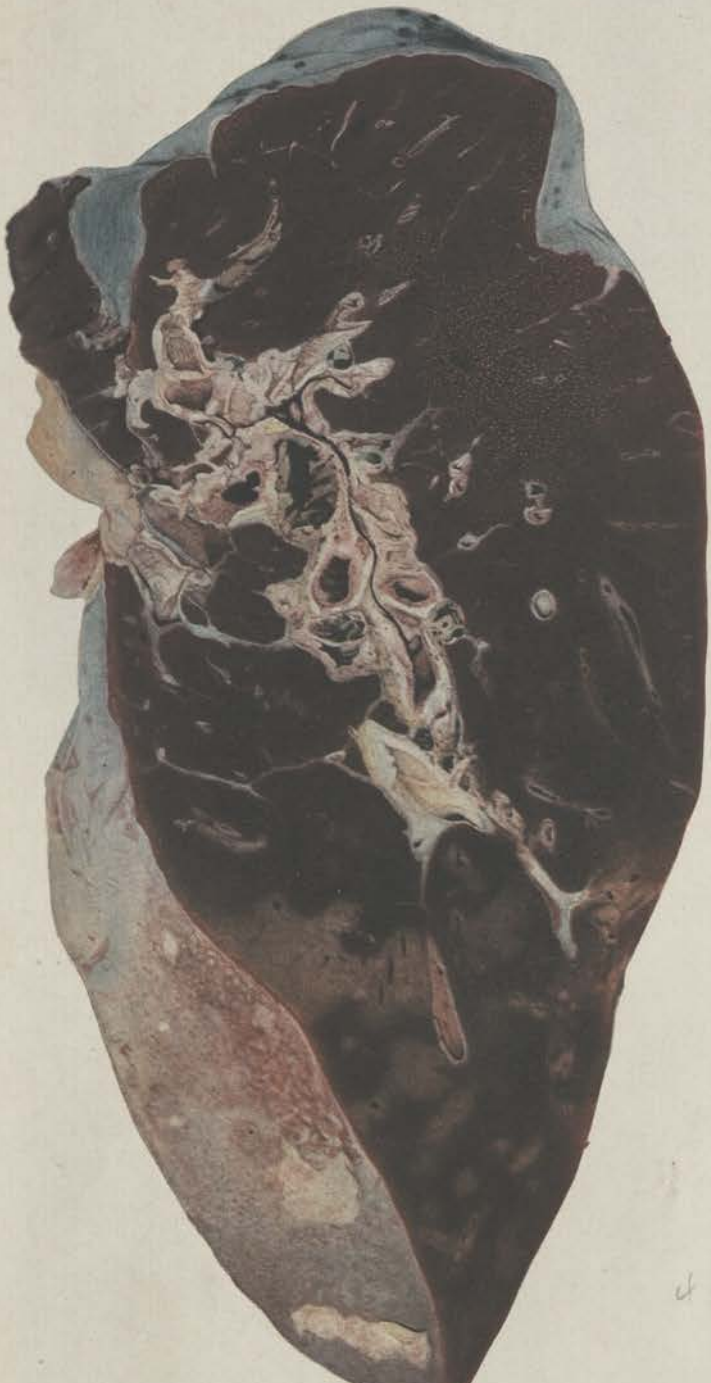


PLATE IX. HUMAN LUNG IN PNEUMONIC PLAGUE, SHOWING MORE WELL-MARKED AREAS OF LOBULAR PNEUMONIA AND PLEURAL EXUDATE.

PLATE X. HUMAN LUNG, PNEUMONIC PLAGUE, SHOWING GRAY HEPATIZATION AND FIBRINOUS PLEURISY.

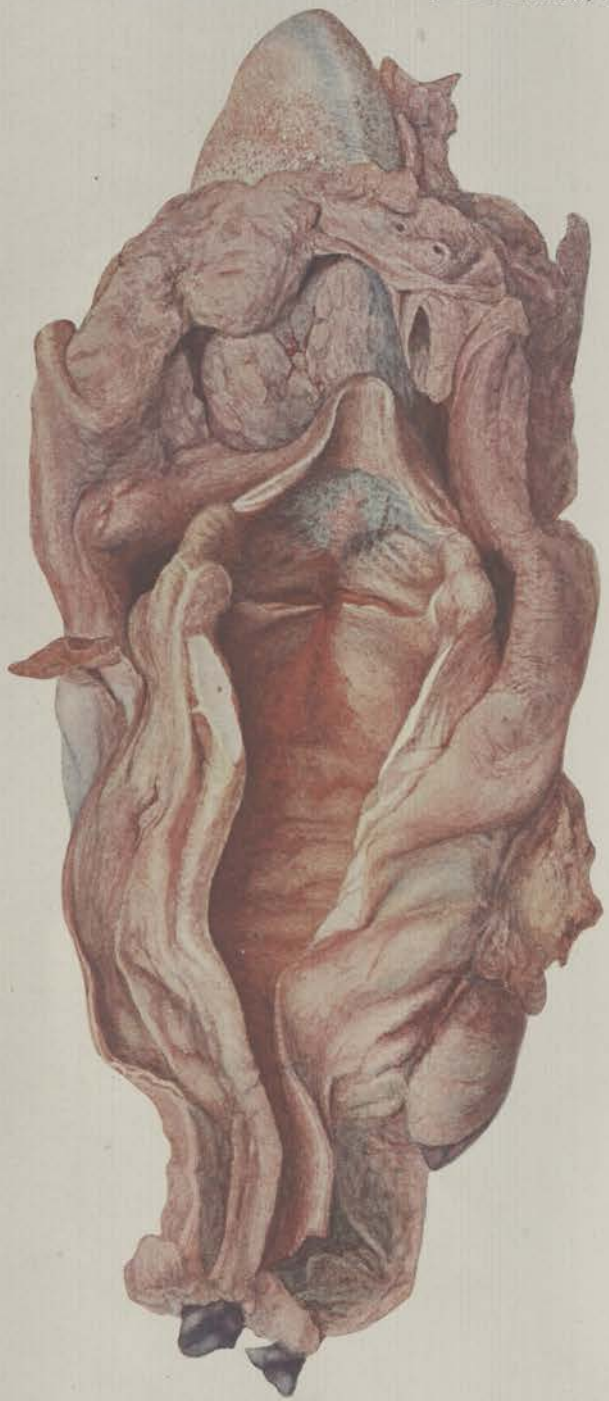


PLATE XI.

PLATE II. THROAT, LARYNX, AND TRACHEA IN PNEUMONIC PLAGUE. MARKED HYPERÆMIA OF THE LARYNX AND TRACHEA; TONSIL NOT SWOLLEN. MARKED HYPERPLASIA OF AN INCISED LYMPHATIC GLAND TO THE RIGHT OF THE TRACHEA, AND OF A SMALL MORE HÆMORRHAGIC LYMPHATIC GLAND AT THE BASE OF THE TRACHEA.

Plate II of the Report of the International Plague Conference is reproduced here as Plate XI.

Fig. 1. Lung of dog with pneumonic plague.

Fig. 2. Liver of tarbagan (*Arctomys bobac* Schreb.), showing chronic plague infection.

PLATE XII.

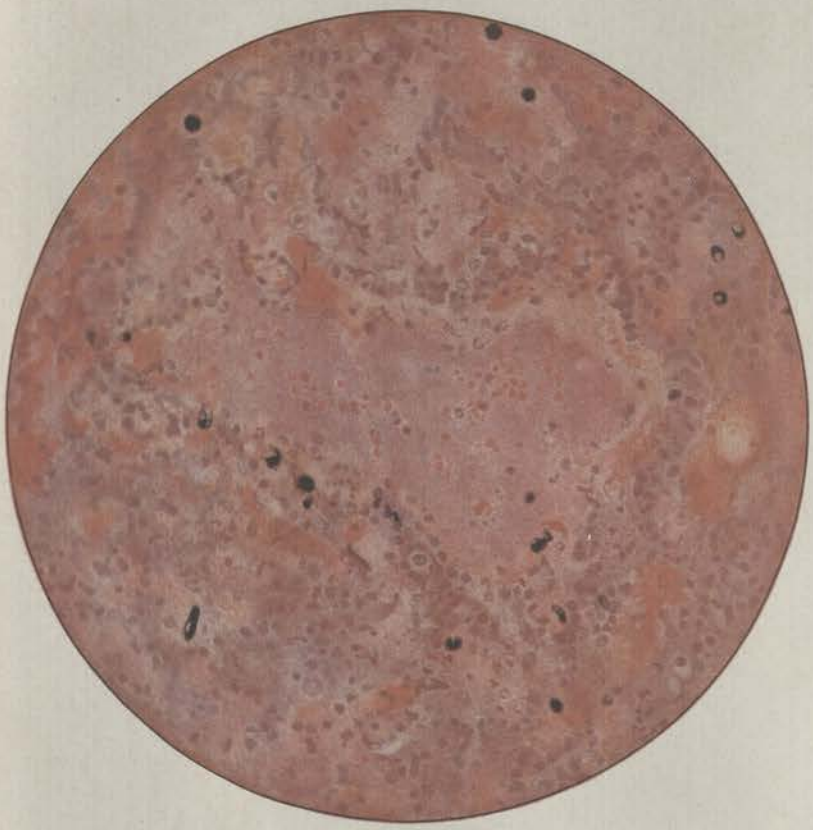


PLATE XIII. MICROSCOPICAL SECTION OF HUMAN LUNG, SHOWING PARTICULARLY CONGESTION OF THE ALVEOLI FILLED WITH PLAGUE BACILLI.

(Drawn from magnification of 150 diameters.)

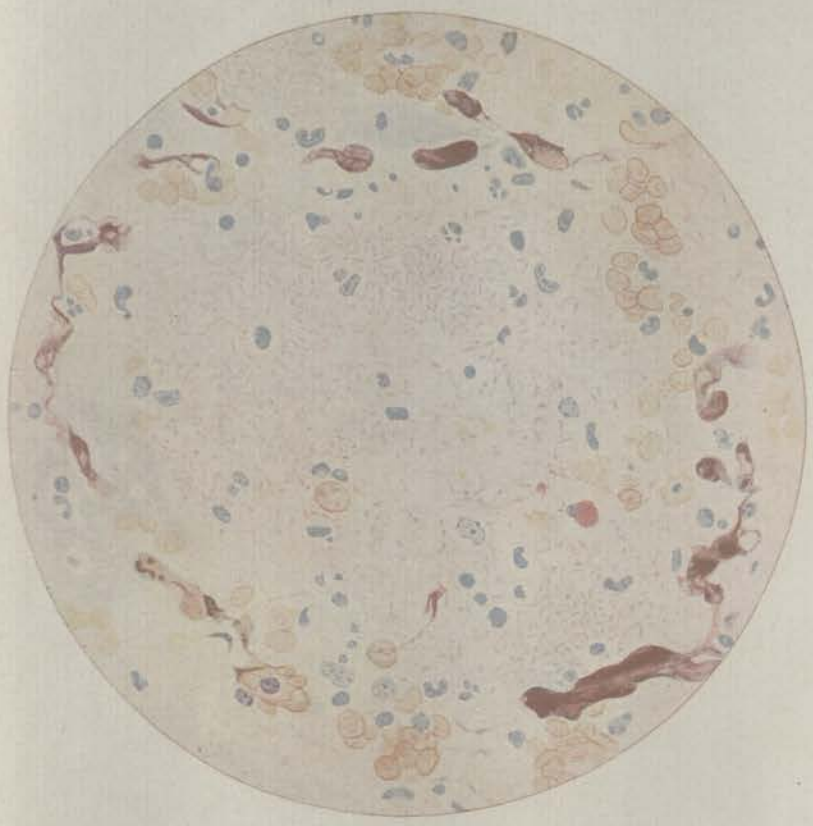


PLATE XIV. MICROSCOPICAL SECTION OF HUMAN LUNG, SHOWING PARTICULARLY ALVEOLI FILLED WITH PLAGUE BACILLI.

(Drawn from magnification of 780 diameters.)

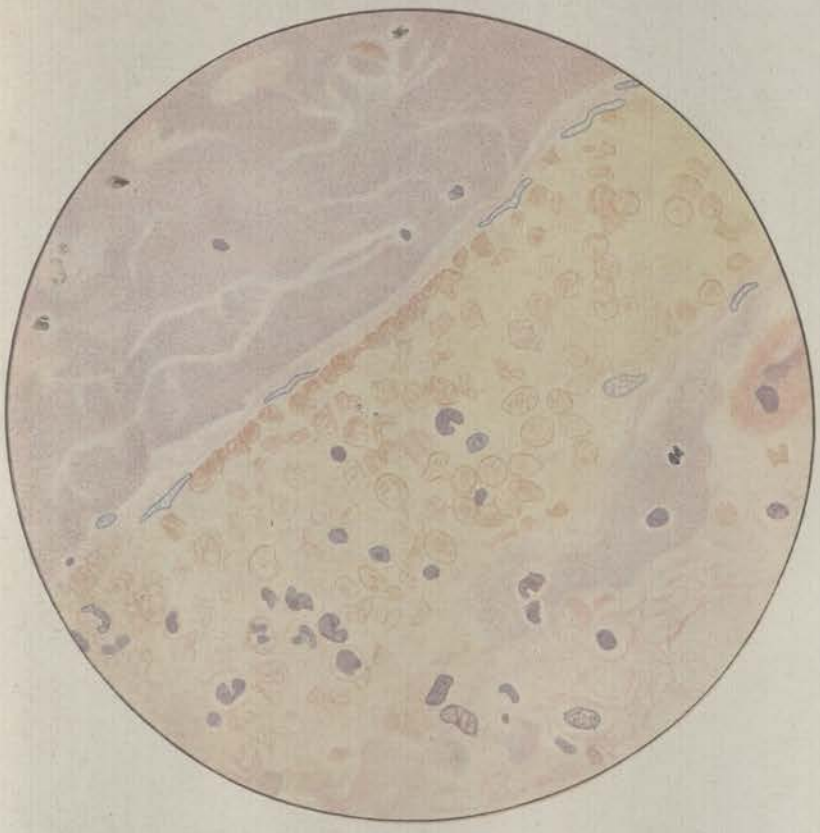


PLATE XV. MICROSCOPICAL SECTION OF HUMAN LUNG IN ADVANCED STAGE OF PNEUMONIA, SHOWING BACILLI IN GREAT MASSES ABOUT BLOOD VESSELS.

(Drawn from magnification of 780 diameters.)

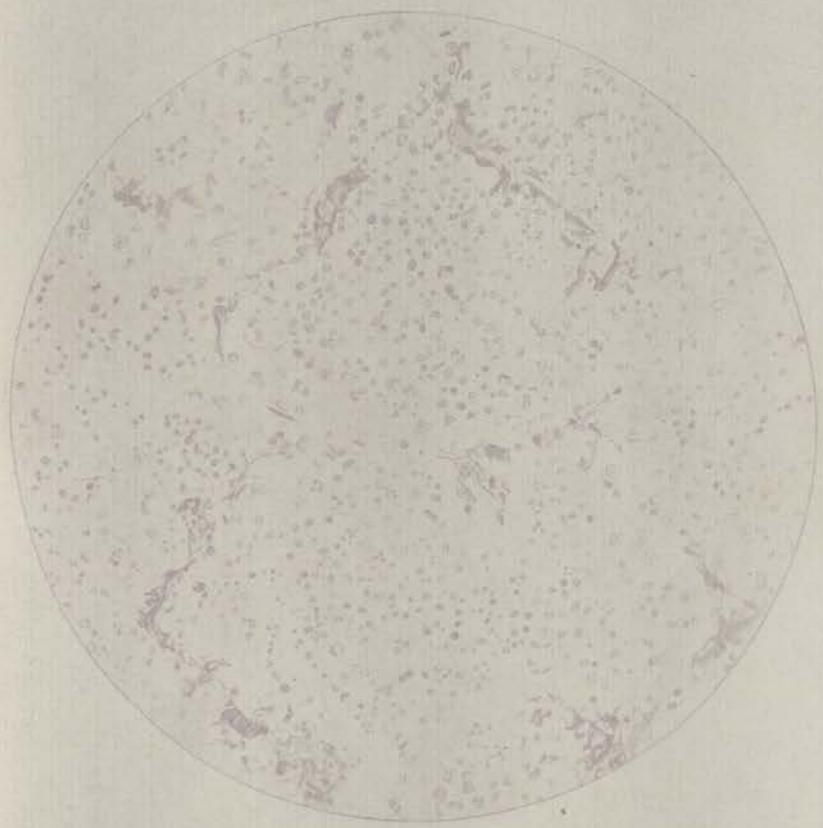


PLATE XVI. MICROSCOPICAL SECTION OF HUMAN LUNG IN STAGE OF HEPATIZATION WITH ABSENCE OF FIBRIN. SECTION STAINED FOR FIBRIN BY WEIGERT'S METHOD.

(Drawn from magnification of 330 diameters.)

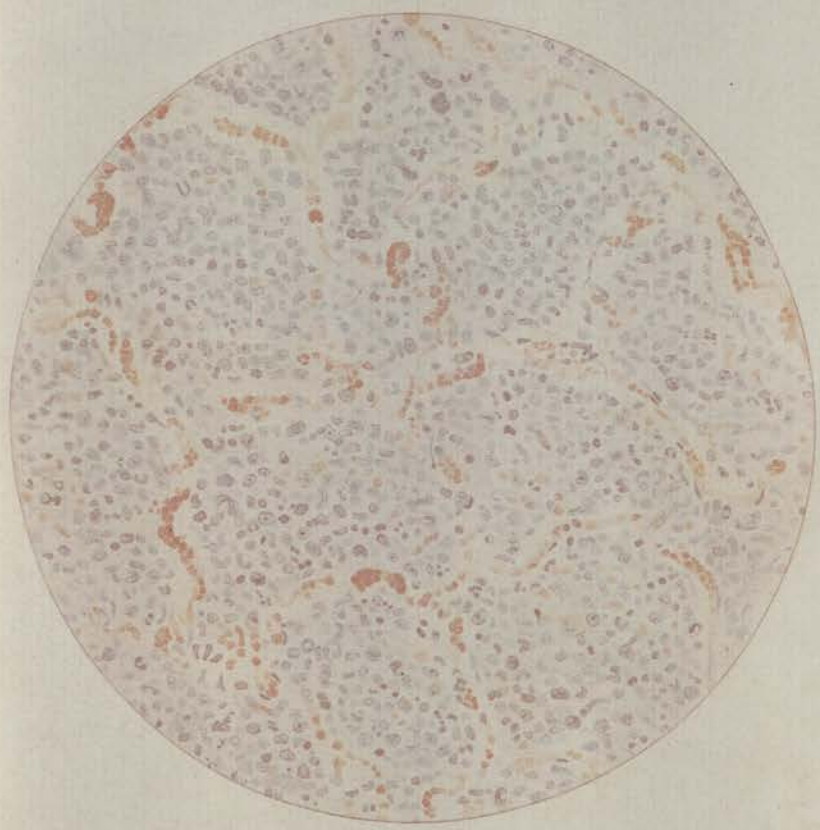


PLATE XVII. MICROSCOPICAL SECTION OF HUMAN LUNG IN PNEUMONIC PLAGUE, SHOWING CHARACTER OF ALVEOLAR EXUDATE.

(Drawn from magnification of 330 diameters.)

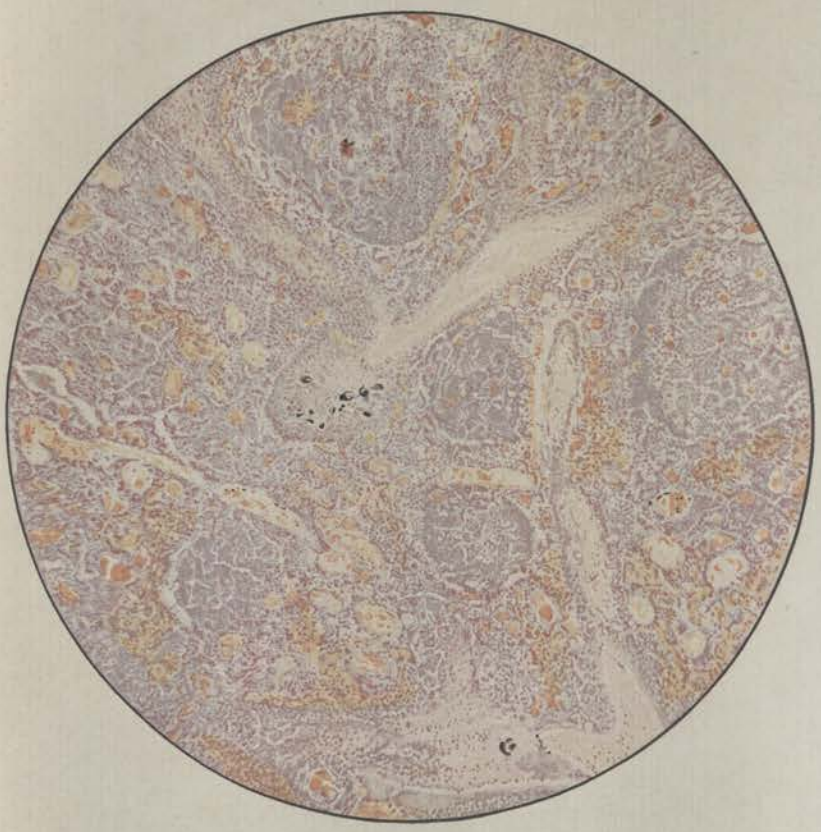


PLATE XVIII. MICROSCOPICAL SECTION OF HUMAN TONSIL, PNEUMONIC PLAGUE, SHOWING CONGESTION.

(Drawn from magnification of 150 diameters.)

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